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No financial support or sponsorship received associated with this presentation by speaker nor speaker's business affiliates and family.



**AAOSH, Nov 2018**

# **Evolutionary Oral Medicine & Probiotics**





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- **Professor**
- **Division of Dentistry**
- **Department of  
Otolaryngology**
- **Feinberg School of  
Medicine**
- **Chicago, IL USA**





# Topics:

- **Probiotics**
- **Diet/Prebiotics**
- **Evolutionary Oral Medicine**
- **Microbiome**
- **Gateway Microbiomes**
- **Barrier Microbiomes**
- **Epigenetics/Epitranscriptomics**





**Nature- Love it or leave it!**

**How man and microbiome evolves...**



**Evolutionary Oral Medicine**



# The “Gateway” Microbiomes

***“Nothing in biology makes sense except  
in the light of evolution.”***

—*Theodosius Dobzhansky*



o  
elial





# Dense Nutrient Food

AMERICAN  
Scientist

From pilfered-from-predators to processed-and-packaged, animals have been part of human diets for more than 3 million years. Cooking was unquestionably a revolution in our dietary history. Cooking makes food both physically and chemically easier to chew and digest, enabling the extraction of more energy from the same amount of food. The biggest increase in brain size in our evolutionary history happened right after we see the earliest evidence for cooking.



# Dental Calculus- Fossils

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## ARTICLE PREVIEW

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NATURE GENETICS | LETTER



[日本語要約](#)

## Sequencing ancient calcified dental plaque shows changes in oral microbiota with dietary shifts of the Neolithic and Industrial revolutions

Christina J Adler, Keith Dobney, Laura S Weyrich, John Kaidonis, Alan W Walker, Wolfgang Haak, Corey J A Bradshaw, Grant Townsend, Arkadiusz Sołtysiak, Kurt W Alt, Julian Parkhill & Alan Cooper





# Dental Calculus- Fossils



postindustrial lifestyles.



# Dental Calculu



Fig. 1. The unquestionable oldest evidence of caries in the Pictures of *H. rhodesiensis* skull cast. Map modified from G

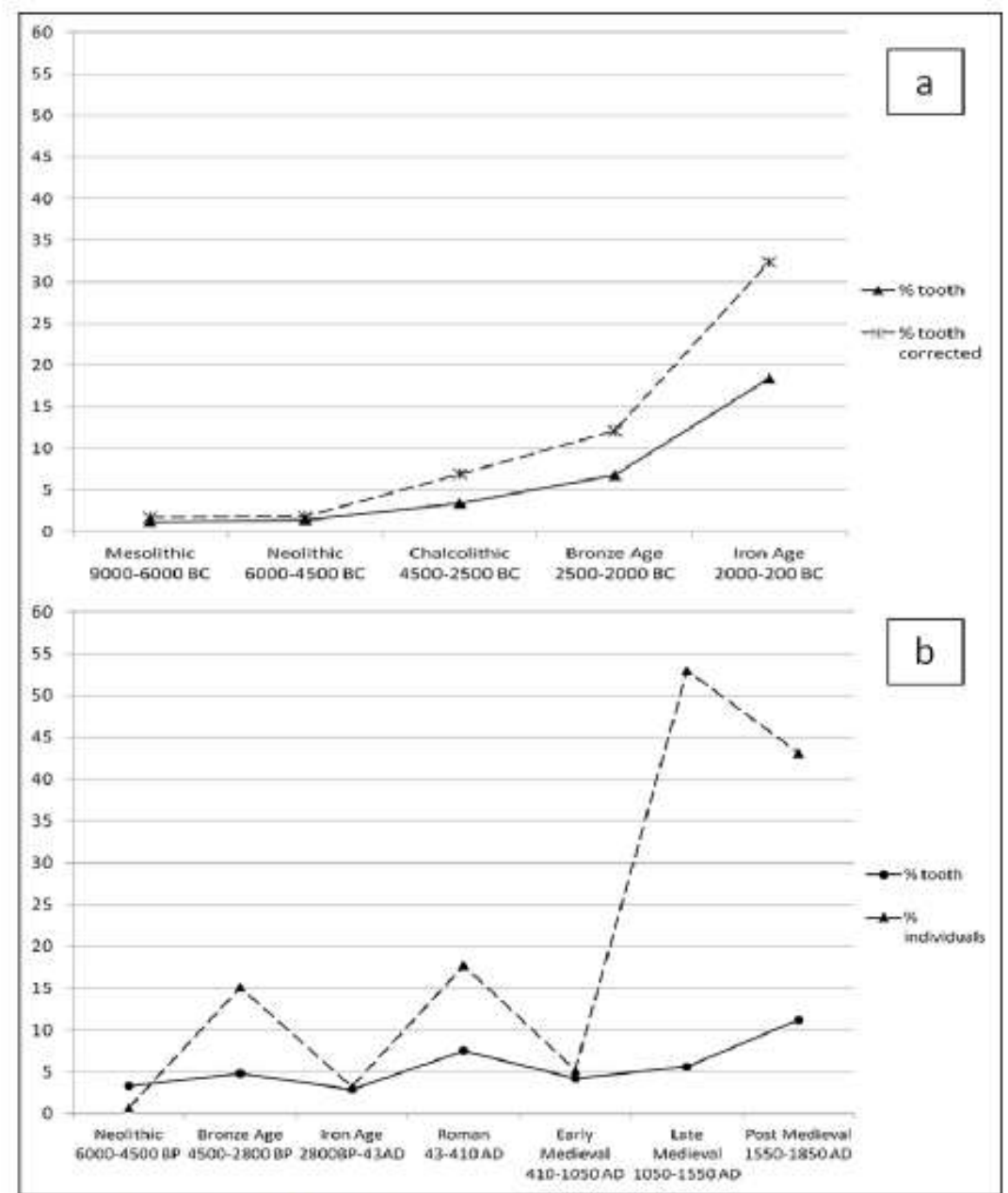


Fig. 2. Caries trends in the Old World across time. a) Indus valley civilization sequence, caries frequency versus corrected frequency (Lukacs, 1996). b) Britain sequence, caries frequency versus prevalence (Roberts & Cox, 2007).



- Shift in microbiome from hunter gather- to farmer –to industrialized age

Nutritional standards of Neolithic populations were generally inferior to that of hunter-gatherers, and that their life expectancy may well have been shorter too, in part due to diseases and harder work. Hunter-gatherers must have covered their food needs with about 20 hours work a week, while agriculture required much more and was at least as uncertain. The hunter-gatherers' diet was more varied and balanced than what agriculture later allowed. Average height went down from 5'10" (178 cm) for men and 5'6" (168 cm) for women to 5'5" (165 cm) and 5'1" (155 cm), respectively, and it took until the twentieth century for average human height to come back to the pre-Neolithic Revolution levels. Agriculturalists had more anemias and vitamin deficiencies, more spinal deformations and more dental pathologies. **HINT!!!! EWS at work!**



# Natufian Period - Hunter-Gatherer Ancestors of Pre-Pottery Neolithic



- The Natufian culture is the name given to the sedentary Late Epi-Paleolithic hunter-gatherers living in the Levant region of the near east between about **12,500 and 10,200** years ago. The Natufians foraged for food such as **emmer wheat, barley and almonds, and hunted gazelle, deer, cattle, horse, and wild boar.**



# Natufian Period - Hunter-Gatherer Ancestors of the Pottery Neolithic

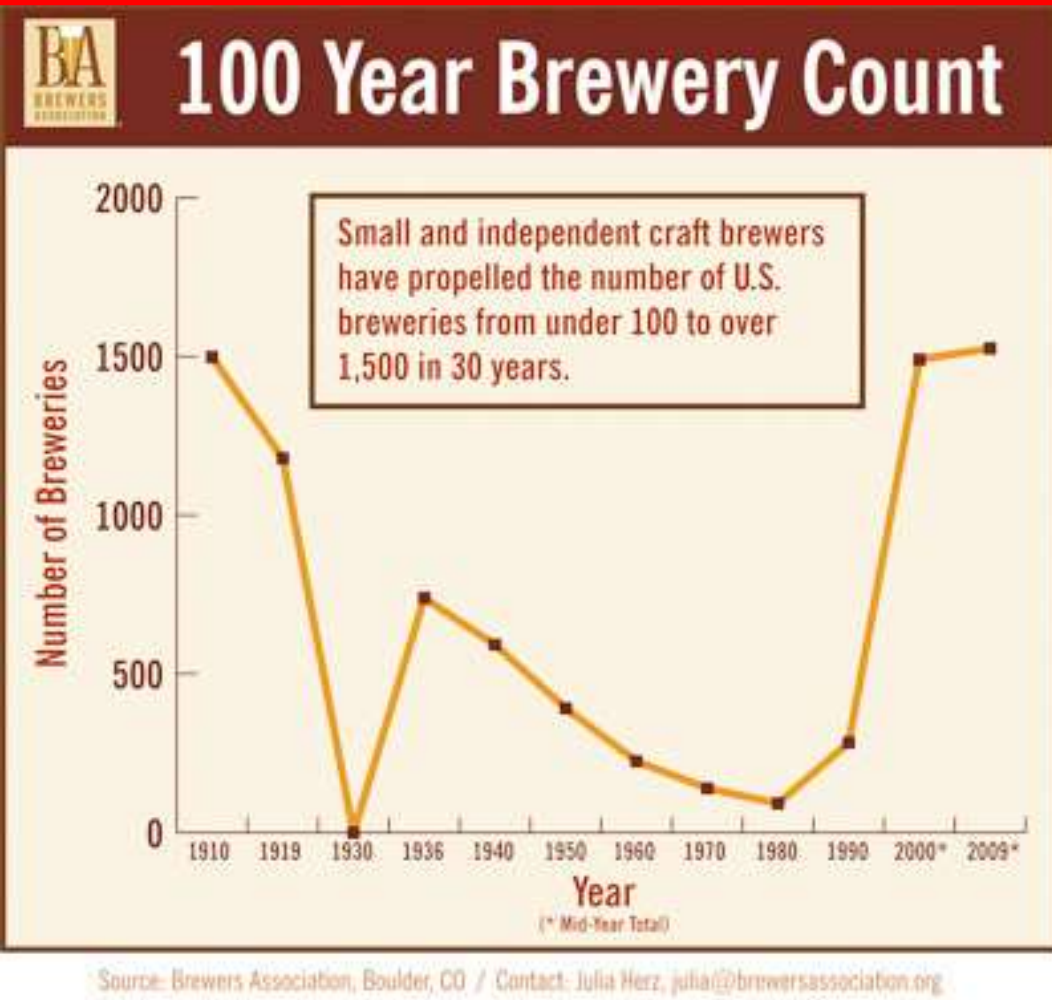


vidence suggests  
**may have cultivated**  
between horticulture  
and agriculture (one and difficult  
cord. **Most scholars**  
**culture was not a**  
**es of experimentation**  
during the Natufian  
nce regimes.





# Natufian Period - Hunter-Gatherer Ancestors of Pre-Pottery Neolithic



source of barley was available.



# Dental Calculus- Fossils

Journal of Human Evolution xxx (2014) 1–6



ELSEVIER

Contents lists available at ScienceDirect

Journal of Human Evolution

journal homepage: [www.elsevier.com/locate/jhevol](http://www.elsevier.com/locate/jhevol)



## Ancient DNA analysis of dental calculus

Laura S. Weyrich<sup>a</sup>, Keith Dobney<sup>b</sup>, Alan Cooper<sup>a,\*</sup>

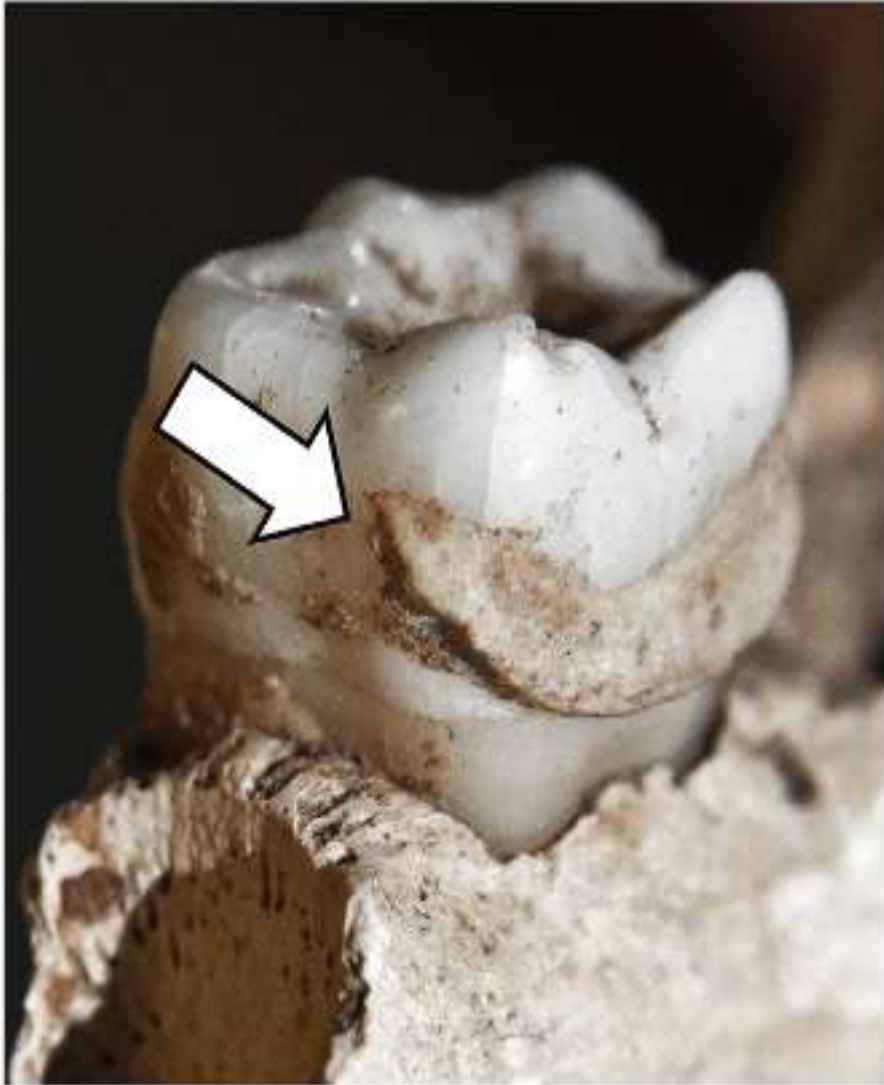
<sup>a</sup> The Australian Centre for Ancient DNA, The University of Adelaide, Adelaide, Australia

<sup>b</sup> Department of Archaeology, School of Geosciences, University of Aberdeen, Aberdeen, UK

- Dental calculus preserves DNA of oral microbiome...



# Dental Calculus-



**Figure 1.** Supra-gingival dental calculus is identifiable in a concave ring on a lower molar from a Medieval specimen, York, UK.





# Dental Calculus- Fossils

- Calcified dental plaque (dental calculus) preserves for millennia and entraps biomolecules from all domains of life and viruses.. **We characterize (i) the ancient oral microbiome in a diseased state, (ii) 40 opportunistic pathogens, (iii) ancient human-associated putative antibiotic resistance genes, (iv) a genome reconstruction of the periodontal pathogen Tannerella forsythia, (v) 239 bacterial and 43 human proteins, allowing confirmation of a long-term association between host immune factors, 'red complex' pathogens and periodontal disease, and (vi) DNA sequences matching dietary sources.** Directly datable and nearly ubiquitous, dental calculus permits the simultaneous investigation of pathogen activity, host immunity and diet, **thereby extending *direct investigation of common diseases into the human evolutionary past.***



# Hominid Microbiome

# Science

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## Cospeciation of gut microbiota with hominids

Andrew H. Moeller<sup>1,2</sup>, Alejandro Caro-Quintero<sup>3</sup>, Deus Mjungu<sup>4</sup>, Alexander V. Georgiev<sup>5,6</sup>, Elizabeth V. Lonsdorf<sup>7</sup>, Martin N. Muller<sup>8</sup>, Anne E. Pusey<sup>9</sup>, Martine Peeters<sup>10</sup>, Beatrice H. Hahn<sup>11</sup>, Howard Ochman<sup>1,\*</sup>

+ Author Affiliations

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Science 22 Jul 2016:  
Vol. 353, Issue 6297, pp. 380-382  
DOI: [10.1126/science.aaf3951](https://doi.org/10.1126/science.aaf3951)





# Hominid Microbiome

- Nuclear, mitochondrial, and gut bacteria genomes diversified in concert during hominid evolution.. past 15 million years

## Abstract

The evolutionary origins of the bacterial lineages that populate the human gut are unknown. Here we show that multiple lineages of the predominant bacterial taxa in the gut arose via cospeciation with humans, chimpanzees, bonobos, and gorillas over the past 15 million years. Analyses of strain-level bacterial diversity within hominid gut microbiomes revealed that clades of Bacteroidaceae and Bifidobacteriaceae have been maintained exclusively within host lineages across hundreds of thousands of host generations. Divergence times of these cospeciating gut bacteria are congruent with those of hominids, indicating that nuclear, mitochondrial, and gut bacterial genomes diversified in concert during hominid evolution. This study identifies human gut bacteria descended from ancient symbionts that speciated simultaneously with humans and the African apes.



- The Hologenome: Microbes drive evolution..



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## Microbes Drive Evolution of Animals and Plants: the Hologenome Concept

Eugene Rosenberg, Ilana Zilber-Rosenberg





- **The hologenome concept of evolution**

“So, like it or not, microbiology is going to be in the center of evolutionary study in the future—and vice versa.”

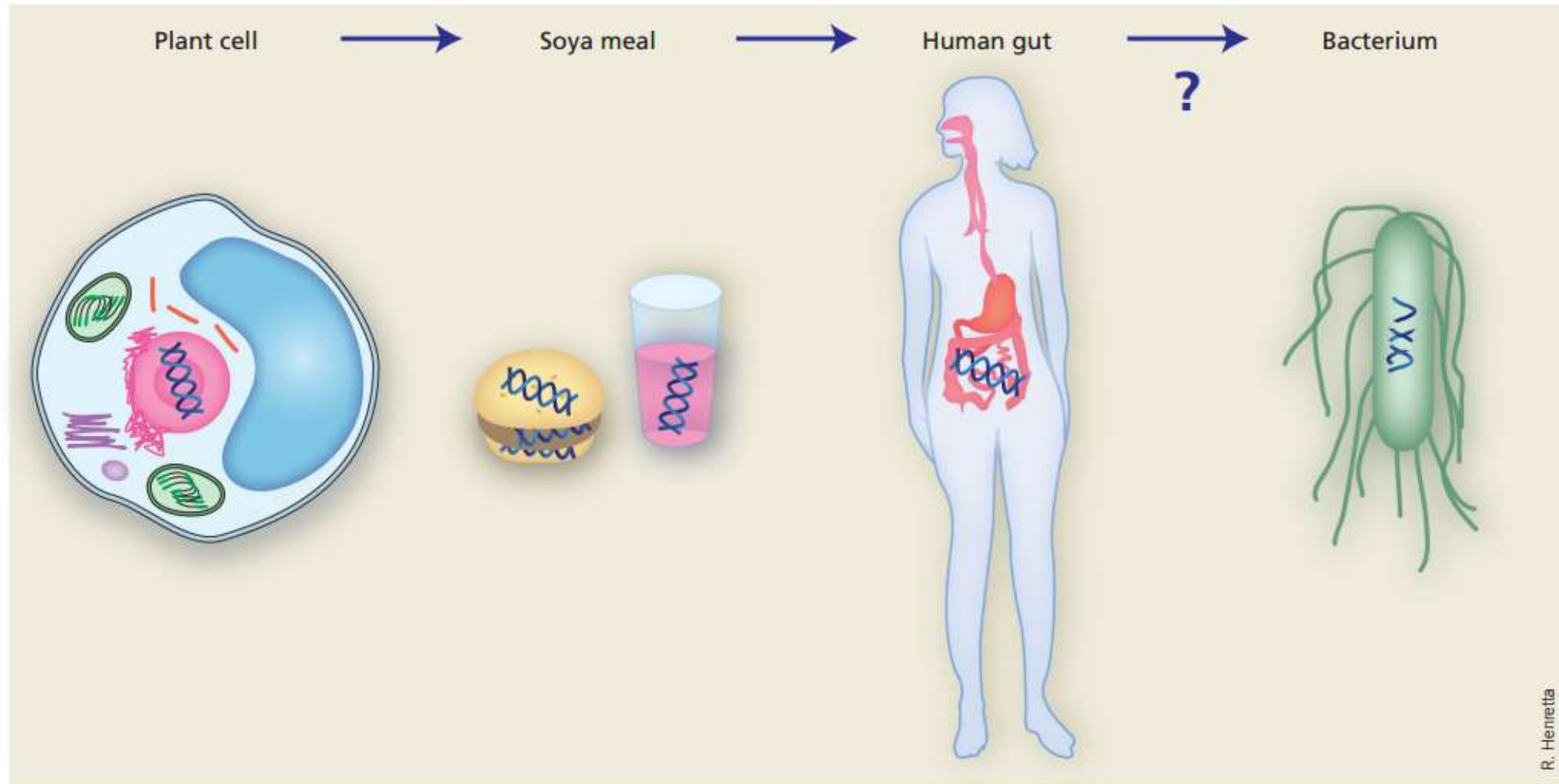
—Carl R. Woese

**viruses.**



# Lateral Gene Transfer

REVISED "TREE" OF LIFE retains and acknowledges that eukaryotes and bacteria. But it also includes an arrow. Those links have been inserted to show the lateral gene transfer of single or multiple genes between different organisms. This "tree" also shows that life probably arose from a common ancestor.



**Figure 1** A possible route for transfer of DNA from plant cells in the human diet to bacteria. Some DNA in food is degraded during cooking and processing, but the remainder is ingested intact. Consumed DNA is largely hydrolyzed during digestion. Netherwood *et al.* provide evidence that intact transgenic DNA can be recovered in the human ileum and taken up by bacteria in this environment.





Research Article/Section III: Case Studies in Extant Primates

## Tubers as fallback foods and their impact on Hadza hunter-gatherers

Frank W. Marlowe , Julia C. Berbesque

Tubers are present within the latitudes that hominins have occupied from their inception through the first expansion of *Homo* out of Africa. Some hominins show dental features (e.g., thick enamel) that have been interpreted as adaptations to eating USOs. Tubers are implicated in the transition to *Homo* and that **cooking is also implicated** because tubers are usually roasted before eaten.

**accessing deep tubers.**



# Tubers and Polyols

In  
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alc  
pho  
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the main glucide  
e. However in a large  
compounds, such as  
orted. Polyols (or sugar  
arose, primary products of  
ude mannitol, sorbitol,  
myo-inositol, ribitol,





# Cellulose and Xylan Hydrolysis

Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa

Carlotta De Filippo<sup>a</sup>, Duccio Cavalieri<sup>a</sup>, Monica Di Paola<sup>b</sup>, Matteo Ramazzotti<sup>c</sup>, Jean Baptiste Poullet<sup>d</sup>, Sebastien Massart<sup>d</sup>, Silvia Collini<sup>b</sup>, Giuseppe Pieraccini<sup>e</sup>, and Paolo Lionetti<sup>b,1</sup>

- Compared the fecal microbiota of European children (EU) and that of children from a rural African village of Burkina Faso (BF), where the diet, high in fiber content, is similar to that of early human settlements at the time of the birth of agriculture. By using high-throughput 16S rDNA sequencing and biochemical analyses, *we found significant differences in gut microbiota between the two groups.*





# Cellulose and Xylan Hydrolysis

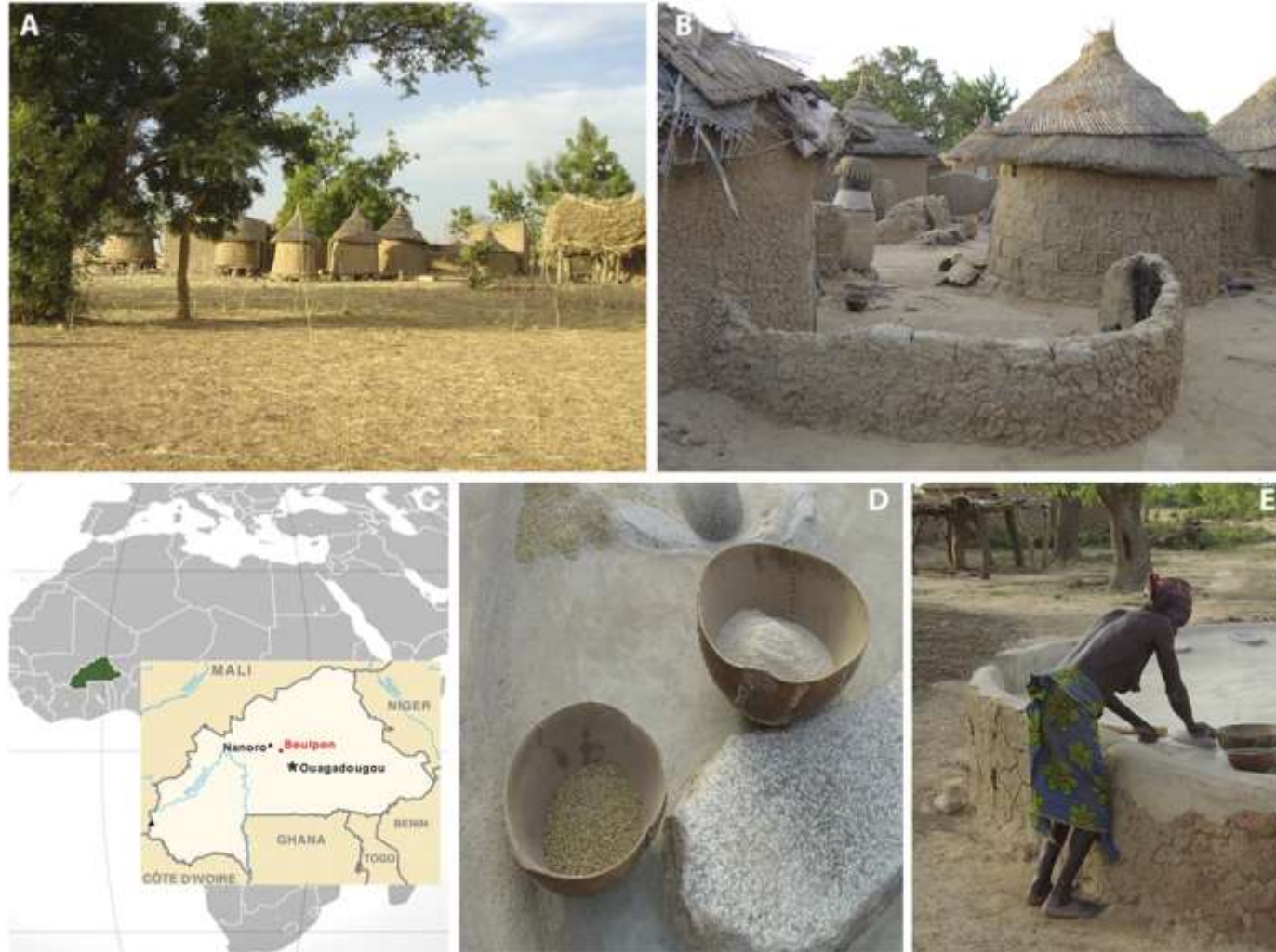
Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa

Carlotta De Filippo<sup>a</sup>, Duccio Cavalieri<sup>a</sup>, Monica Di Paola<sup>b</sup>, Matteo Ramazzotti<sup>c</sup>, Jean Baptiste Poullet<sup>d</sup>, Sebastien Massart<sup>d</sup>, Silvia Collini<sup>b</sup>, Giuseppe Pieraccini<sup>e</sup>, and Paolo Lionetti<sup>b,1</sup>

- BF children showed a significant enrichment in Bacteroidetes and depletion in Firmicutes ( $P < 0.001$ ), with a unique abundance of bacteria from the genus Prevotella and Xylanibacter, known to contain a set of bacterial genes for cellulose and xylan hydrolysis, completely lacking in the EU children.



## Life in a rural village of Burkina Faso.



Carlotta De Filippo et al. PNAS 2010;107:14691-14696



# Cellulose and Xylan Hydrolysis

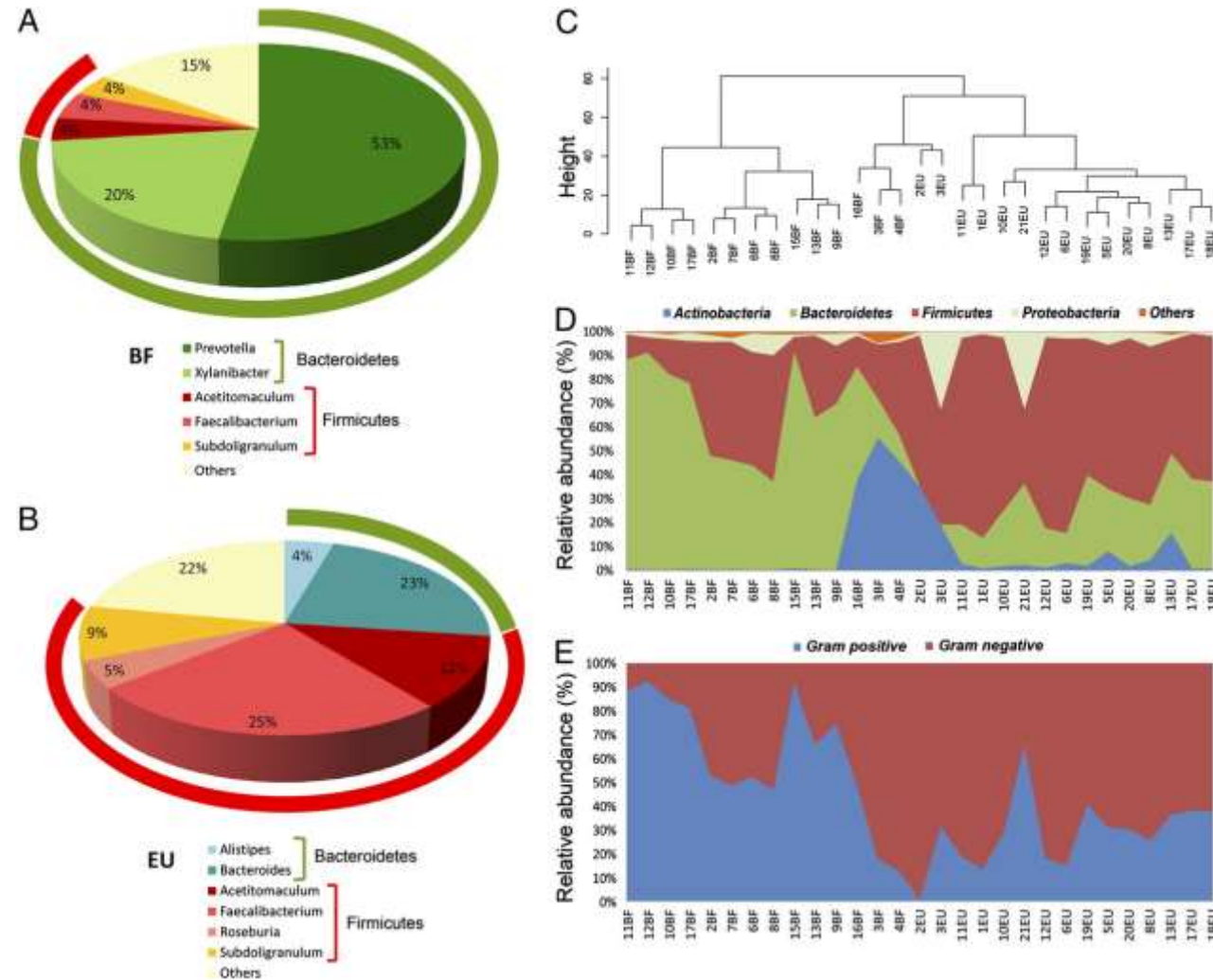


frica



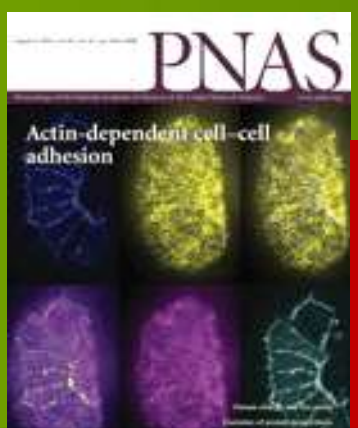


# 16S rRNA gene surveys reveal a clear separation of two children populations investigated.



Carlotta De Filippo et al. PNAS 2010;107:14691-14696





## Cellulose and Xylan Hydrolysis

Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa

Carlotta De Filippo<sup>a</sup>, Duccio Cavalieri<sup>a</sup>, Monica Di Paola<sup>b</sup>, Matteo Ramazzotti<sup>c</sup>, Jean Baptiste Poullet<sup>d</sup>,

When does the protection start? How do children develop the protective microbiome?  
Before birth!





# Pre-natal microbiome

- The Maternal Effect- everyone knows!

BE

SCIENTIFIC  
AMERICAN

English ▾ Cart  Sign in

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HEALTH

# How Bacteria in the Placenta Could Help Shape Human Health

The placenta is full of microbes, a new study finds, raising questions about how that ecosystem and mothers' oral health influence the risk of preterm birth

---

By Dina Fine Maron on May 21, 2014

 2





# Pre-natal microbiome

- **The Maternal Effect**



Science  
Translational  
Medicine

Vol 6, Issue 237  
21 May 2014

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## The Placenta Harbors a Unique Microbiome

Kjersti Aagaard<sup>1,2,3,\*</sup>, Jun Ma<sup>1,2</sup>, Kathleen M. Antony<sup>1</sup>, Radhika Ganu<sup>1</sup>, Joseph Petrosino<sup>4</sup> and James Versalovic<sup>5</sup>

+ See all authors and affiliations

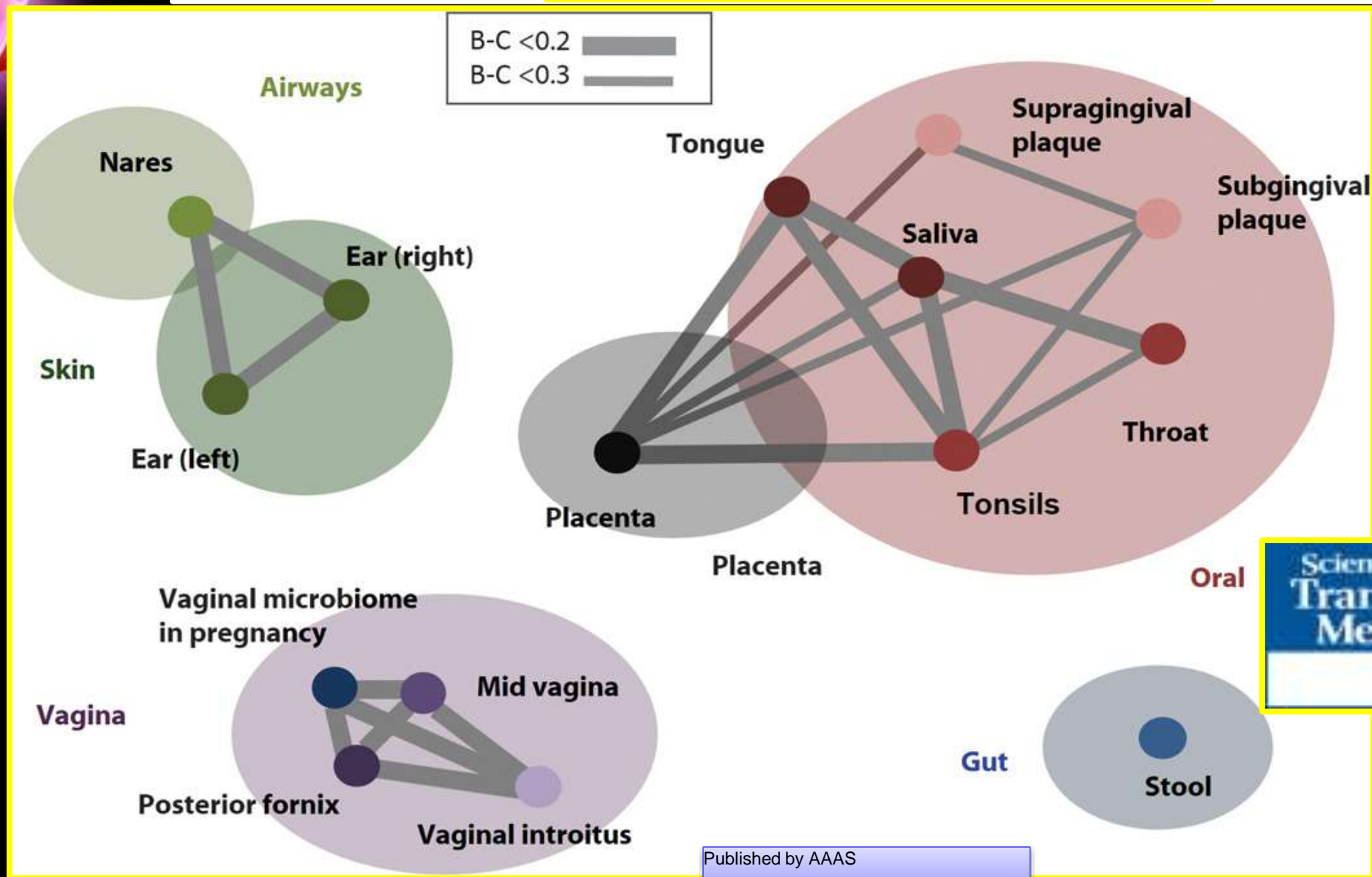
*Science Translational Medicine* 21 May 2014:  
Vol. 6, Issue 237, pp. 237ra65  
DOI: 10.1126/scitranslmed.3008599

We characterized a unique placental microbiome niche, composed of nonpathogenic commensal microbiota from the Firmicutes, Tenericutes, Proteobacteria, Bacteroidetes, and Fusobacteria phyla. **In aggregate, the placental microbiome profiles were most akin (Bray-Curtis dissimilarity <0.3) to the human oral microbiome.** 16S-based operational taxonomic unit analyses revealed associations of the placental microbiome with a remote history of antenatal infection (permutational multivariate analysis of variance,  $P = 0.006$ ), such as urinary tract infection in the first trimester, as well as with preterm birth <37 weeks ( $P = 0.001$ ).





**Fig. 1. The placental microbiome has a taxonomic profile that is similar to the oral microbiome.**





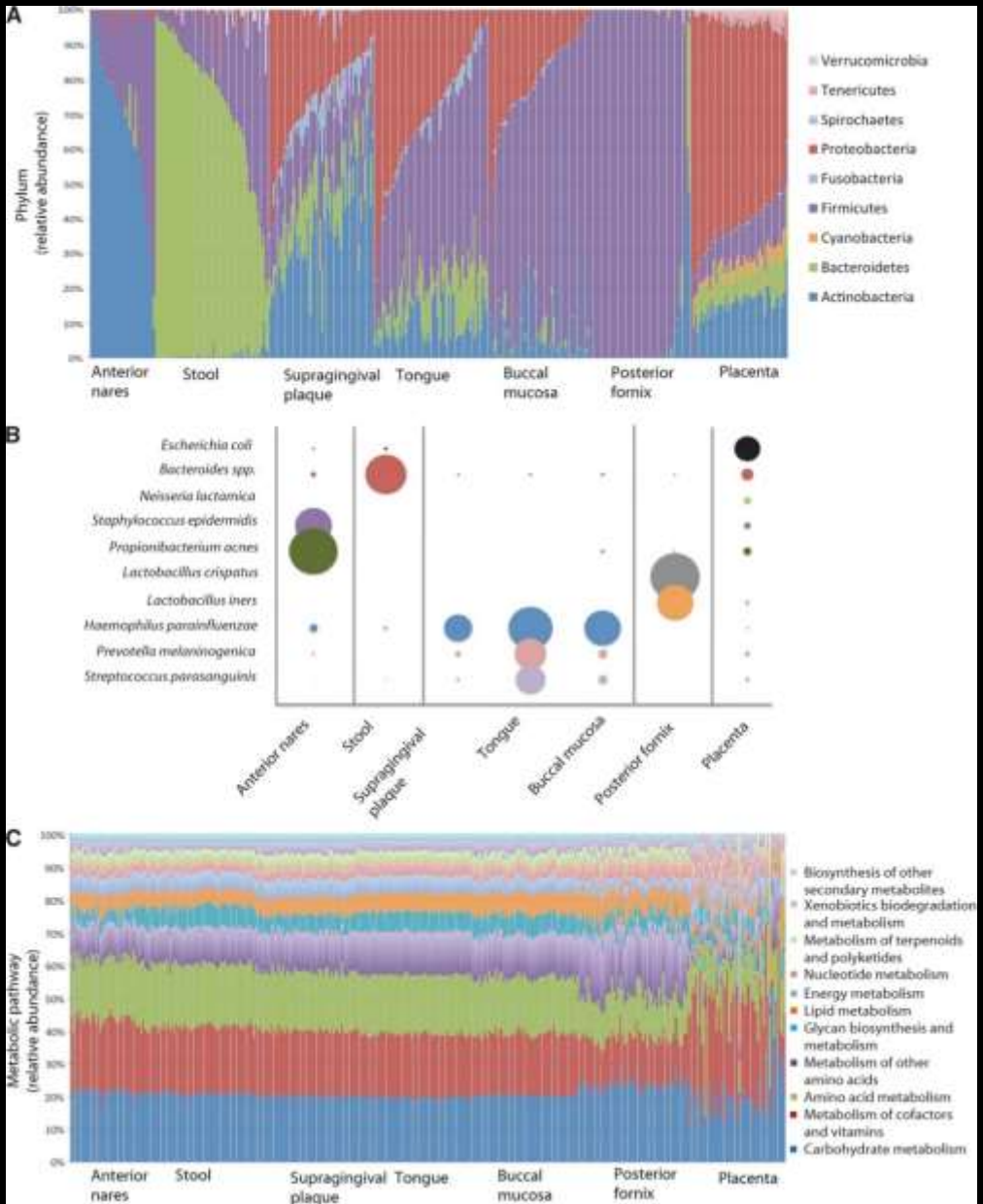


**Fig. 2. Comparison of WGS-generated taxa and metabolic capacity among body sites reveals distinct features of the placental microbiome.**

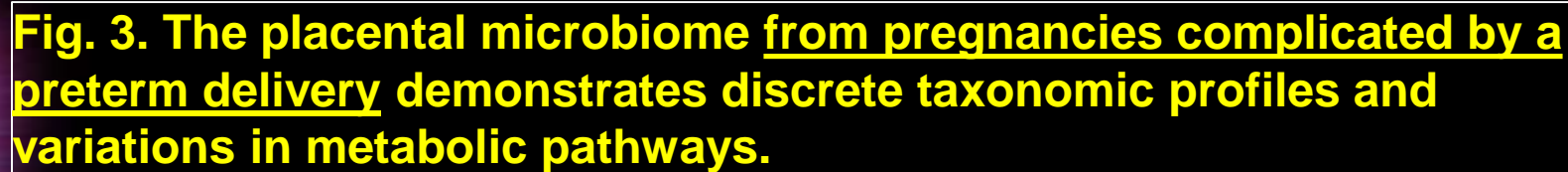


Published by AAAS

# Placenta distinct



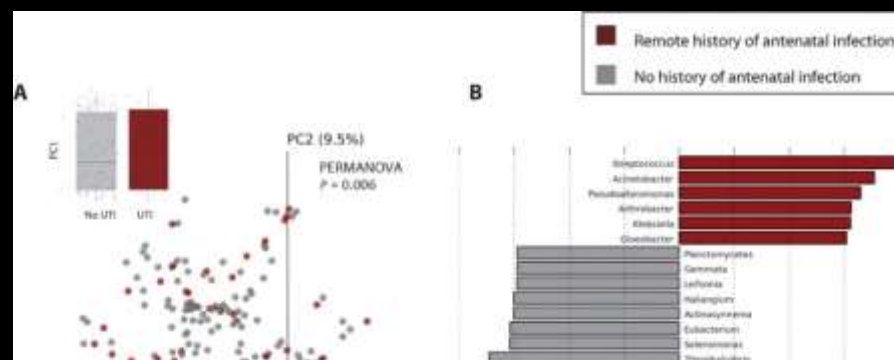




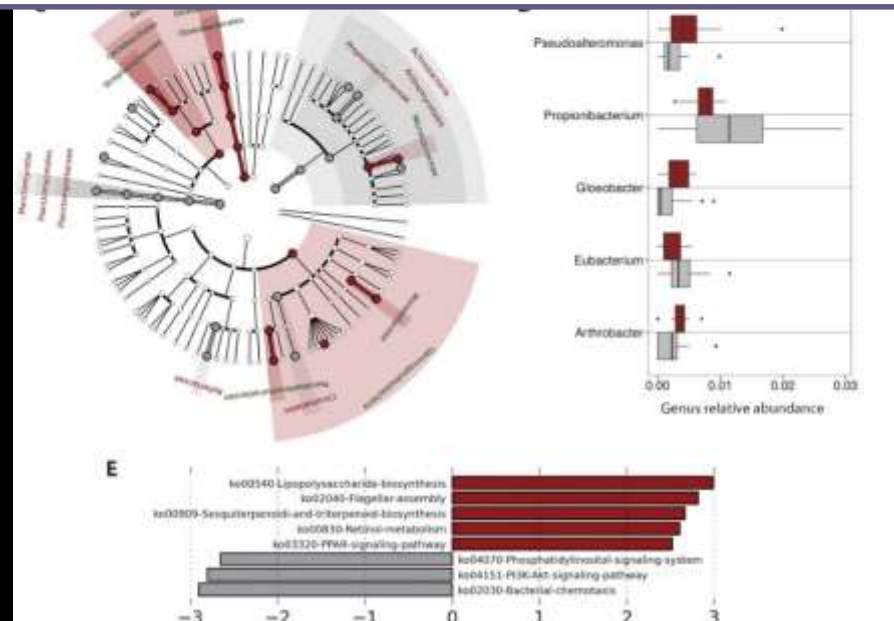




**Fig. 4. A remote history of maternal antenatal infection correlates with the placental microbiome community.**



**Antibiotics again!**







# We change our microbiome with:

antibiotics  
climate  
environment  
diet  
medications  
lack of exercise  
C-sections  
disinfectants

**“The Hygiene Hypothesis”**



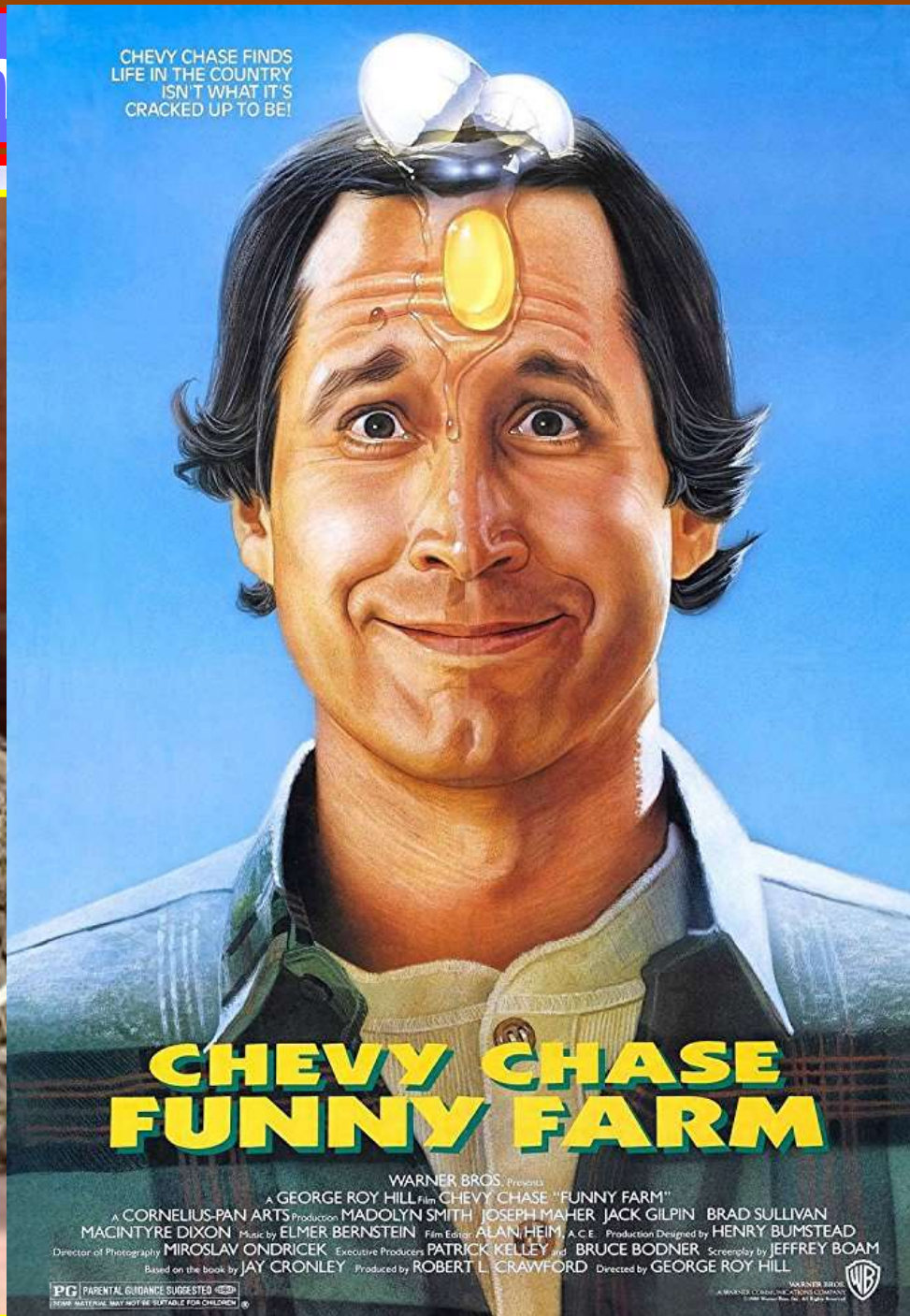
# Take Home- probiotics



- We all have Dysbiosis due to antibiotics, anti-microbials, preservatives, environment and diet. All of it. All of us. Everything is changing.



# Chicken



The first land-dwelling animals were four-legged amphibians that left the water around 360 million years ago



# Washing Eggs



## Shifts in Bacterial Communities of Eggshells and Antimicrobial Activities in Eggs during Incubation in a Ground-Nesting Passerine

Stéphanie Grizard , Maaïke A. Versteegh, Henry K. Ndithia, Joana F. Salles, B. Irene Tieleman

Published: April 16, 2015 • <https://doi.org/10.1371/journal.pone.0121716>

Microbial invasion of egg contents is a cause of embryonic death. To counter infection risks, **the embryo is protected physically by the eggshell and chemically by antimicrobial proteins.** If microbial pressure drives embryo mortality, then females may have evolved, through natural selection, to adapt their immune investment into eggs. Lastly, our results provide limited evidence of significant correlation between antimicrobial compounds and bacterial communities. Our study examined simultaneously, for the first time in a wild bird, **the dynamics of bacterial communities present on eggshells and of albumen-associated antimicrobial components during incubation** and investigated their relationship.







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Washing Eggs- Good idea?



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Kids  
and  
Donuts? ??

2/3 more  
times  
-6 times





# Sugar and Children

## ACTA PÆDIATRICA NURTURING THE CHILD

Regular Article

**Longitudinal birth cohort study found that a significant proportion of children had abnormal metabolic profiles and insulin resistance at 6 years of age**

Emma Kjellberg✉, Josefine Roswall, Stefan Bergman, Gerd Almqvist-Tangen, Bernt Alm, Jovanna Dahlgren

First published: 17 October 2018 | <https://doi.org/10.1111/apa.14599>

- **A significant percentage of 6-year-old children showed abnormal metabolic profiles**, including insulin resistance, which increased their risk of cardiovascular disease. Waist circumference was a stronger marker for metabolic alterations than body mass index.





# Sugar and the AAPD



## Diet Quality, Added Sugar, and Dietary Fiber Intakes in American Preschoolers

**Authors:** Kranz, Sibylle; Smiciklas-Wright, Helen; Francis, Lori A.

**Source:** Pediatric Dentistry, Volume 28, Number 2, March/April 2006, pp. 164-171(8)

**Publisher:** American Academy of Pediatric Dentistry

- 2006- Back when we worried about sugar!
- Lack of fibers, prebiotics and tons of sugar





# Sugar Linked To Decay



J Dent Res. 2014 Jan; 93(1): 8–18.

doi: [10.1177/0022034513508954](https://doi.org/10.1177/0022034513508954)

## Effect on Caries of Restricting Sugars Intake Systematic Review to Inform WHO Guidelines

P.J. Moynihan<sup>1,\*</sup> and S.A.M. Kelly<sup>2</sup>



# War Rationing

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[Home](#) > [UCSF News Center](#) > Sugar Papers Reveal Industry Role in Shifting National Heart Disease Focus to Saturated Fat

## Sugar Papers Reveal Industry Role in Shifting National Heart Disease Focus to Saturated Fat

### BILL OF HEALTH

All the good things you've always wanted to believe about sugar are true. And the government has confirmed them. An extensive study by the Food and Drug Administration has demolished the myths about natural sugar once and for all.

The FDA has confirmed that:

☒ Sugar is not the cause of obesity.

#### How About Dental Health?

Cavities are declining dramatically, primarily due to the use of fluoride. The FDA report points out that all carbohydrates, not just sugar, can contribute to cavities. But only when left on the teeth. Proper dental hygiene is a strong deterrent to tooth decay.

Not Sugar

COMPULSORY RATION  
of Sugar  
per month  
ration



# Sugar and Fluoride



SEPTEMBER, 1960



ELMWOOD DENT

Dental report for: *Jeffrey*

*Not one new*

*Jeffrey this time*

*MO*

Norman Rockwell

“Look, Mom—no cavities!”

Crest Toothpaste means far fewer cavities for all the family. And Crest freshens your mouth and sweetens your breath.



Manufactured by P. D. Colman and Son, Inc., New York, N.Y. © 1960, P. D. Colman and Son, Inc.

BRING ON THE CANDY

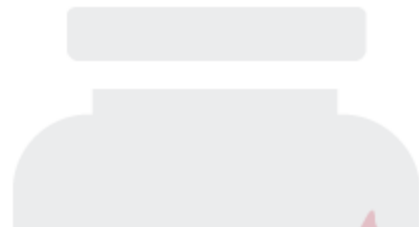
# Crest HAS YOU COVERED







## U.S. CANDY SALES

EVERDAY VS.  
SEASONAL

DOLLAR SALES

DOLLARS % CHANGE VS.  
PREVIOUS 52 WEEKSTOTAL CANDY SALES  
(EVERYDAY & SEASONAL)

\$21,509,317,710

 2.6%

“We donate our candy to the homeless”... OMG totally clueless!!

Copyright © 2016 The Nielsen Company

- Candy sales go up every year! Confectionary sales are at \$38.9 billion. CREST is winning!





P.J. Moynihan<sup>1,\*</sup> and S.A.M. Kelly<sup>2</sup>

# “Fluoride delays decay...”

- Many studies support this, but has led to a decline in dental caries. nonetheless, dental caries has increased. it has been suggested that early dental lesions to a later age shows that, despite the reduction in sugars and dental caries (Marthaler, 1990; Holt, 1990; and so many other references).



important factor that  
merica, and Australia;  
adults). **However,**  
**cavitation**  
of identified studies  
**onship between**  
et al., 1988;  
nzel and Fischer,





# Water Fluoridation

## Water Fluoridation and Dental Caries in U.S. Children and Adolescents

G.D. Slade, W.B. Grider, W.R. Maas, A.E. Sanders

First Published June 14, 2018 | Research Article |



<https://doi.org/10.1177/0022034518774331>

Journal of Dental Research



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action





# Tooth brushing- JDR Oct. 2016

## Clinical Review

### Effect of Toothbrushing Frequency on Incidence and Increment of Dental Caries: A Systematic Review and Meta-Analysis

Journal of Dental Research  
2016, Vol. 95(11) 1230–1236  
© International & American Associations  
for Dental Research 2016  
Reprints and permissions:  
sagepub.com/journalsPermissions.nav  
DOI: 10.1177/0022034516655315  
jdr.sagepub.com

S. Kumar<sup>1</sup>, J. Tadakamadla<sup>1</sup>, and N.W. Johnson<sup>2</sup>

#### Abstract

Toothbrushing is considered fundamental self-care behavior for maintenance of oral health, and brushing twice a day has become a social norm, but the evidence base for this frequency is weak. This systematic review and meta-analysis aims to assess the effect of toothbrushing frequency on the incidence and increment of carious lesions. Medline, Embase, Cinahl, and Cochrane databases were searched. Screening and quality assessment were performed by 2 independent reviewers. Three different meta-analyses were conducted: 2 based on the caries outcome reported in the studies (incidence and increment) with subgroup analyses of categories of toothbrushing frequency; another included all studies irrespective of the caries outcome reported with the type of dentition as subgroups. Meta-regression was conducted to assess the influence of sample size, follow-up period, diagnosis level for carious lesions, and methodological quality of the articles on the effect estimate. Searches retrieved 5,494 titles: after removing duplicates, 4,305 remained. Of these, 74 were reviewed in full, but only 33 were eligible for inclusion. Self-reported infrequent brushers demonstrated higher incidence (odds ratio [OR], 1.50; 95% confidence interval [CI], 1.34 to 1.69) and increment (standardized mean difference [SMD], 0.28; 95% CI: 0.13 to 0.44) of carious lesions than frequent brushers. The odds of having carious lesions differed little when subgroup analysis was conducted to compare the incidence between  $\geq 2$  times/d vs  $< 2$  times/d (OR: 1.45; 95% CI: 1.21 to 1.74) and  $\geq 1$  time/d vs  $< 1$  time/d brushers (OR: 1.56; 95% CI: 1.37 to 1.78). When meta-analysis was conducted with the type of dentition as subgroups, the effect of infrequent brushing on incidence and increment of carious lesions was higher in deciduous (OR: 1.75; 95% CI: 1.49 to 2.06) than permanent dentition (OR: 1.39; 95% CI: 1.29 to 1.49). Findings from meta-regression indicated that none of the included variables influenced the effect estimate.

**Keywords:** dentition, home care dental devices, oral hygiene, preventive dentistry, public health, epidemiology





# Value of Fluoride Toothpaste?

“Individuals who state that they brush their teeth infrequently are at greater risk for the incidence or increment of new carious lesions than those brushing more frequently. The effect is more pronounced in the deciduous than in the permanent dentition.

A few studies indicate that this effect is **independent of the presence of fluoride in toothpaste.**

It is also possible that other factors in those claiming a higher frequency of brushing, such as greater health awareness and motivation, higher socioeconomic status, and a healthier diet, are responsible for the observed effects.”







# Fluoride Delays Decay ?

Figure 2. Prevalence of dental origin: United States, 2011–20

Dental caries experience <sup>1</sup>	
Total	
65–74	
75 and over	
Non-Hispanic white	
Non-Hispanic black	
Hispanic	
Non-Hispanic Asian	
Untreated dental caries	
Total	
65–74	
75 and over	
Non-Hispanic white	
Non-Hispanic black	
Hispanic	
Non-Hispanic Asian	

<sup>1</sup>Includes untreated and treated (restored)  
<sup>2</sup>Significantly different from non-Hispanic  
<sup>3</sup>Significantly different from Hispanic adul  
<sup>4</sup>Significantly different from non-Hispanic  
NOTE: Access data table for Figure 2 at:  
SOURCE: CDC/NCHS, National Health a



Hispanic





# Know thine enemy

## “6 Ways to Reduce Your Child’s Sugary Snacking”



Brought to you by the  
**ADA** American Dental Association®

LIFE STAGES

ADA SEAL  
PRODUCTS

## WHAT DOES 50 GRAMS OF SUGAR LOOK LIKE?

The FDA has proposed a limit on daily sugar consumption of **10 percent** of daily calories – meaning no more than **50 grams** of added sugar per day.

That's less sugar than one bottle of Coke.

So what meals could you make that contain **less than 50g of sugar**?



**COCA-COLA**  
16 fl. oz.  
52 grams sugar



?



# ADA- What is missing?

## Suggestions!!! And health



what we buy changes over time as better products became available, i.e. non GMO-





# Dr. Robert Lustig-UCSF



- **Robert H. Lustig, M.D.**, MSL, is Professor of Pediatrics in the Division of Endocrinology and a member of the Institute for Health Policy Studies at University of California, San Francisco. He has authored 120 peer-reviewed articles and 70 reviews.





**YOU'RE FAT**

**DON'T SUGARCOAT IT  
CAUSE YOU'LL EAT THAT TOO**

gar

“Sugar is now  
been added to  
choice and the  
6,000,000 co  
added caloric

- Dr. Robert

“Fat Chance  
Food, Obesi

ide, and has  
ng consumer  
**percent** of the  
States have

Processed





# Sugar- Pure, White, and Deadly



Sugar for kids= alcohol







# World Obesity

- Thank God we aren't winning!

The world is round

Obesity prevalence

— United Arab Emirates



1980 85 90

Source: Gretchen Steiner  
Population Health Monitor

**I DON'T SMOKE  
WEED...**

**I DEEP FRY IT!**





# Mexico and Sugar

- Mexican children have also related to higher E approximately **73% of adult males are over** important role in the qu activity patterns of their lifestyle patterns from p **potential, 73% are ov** **mothers breastfeed t**



Coke: 0



Pepsi: 1

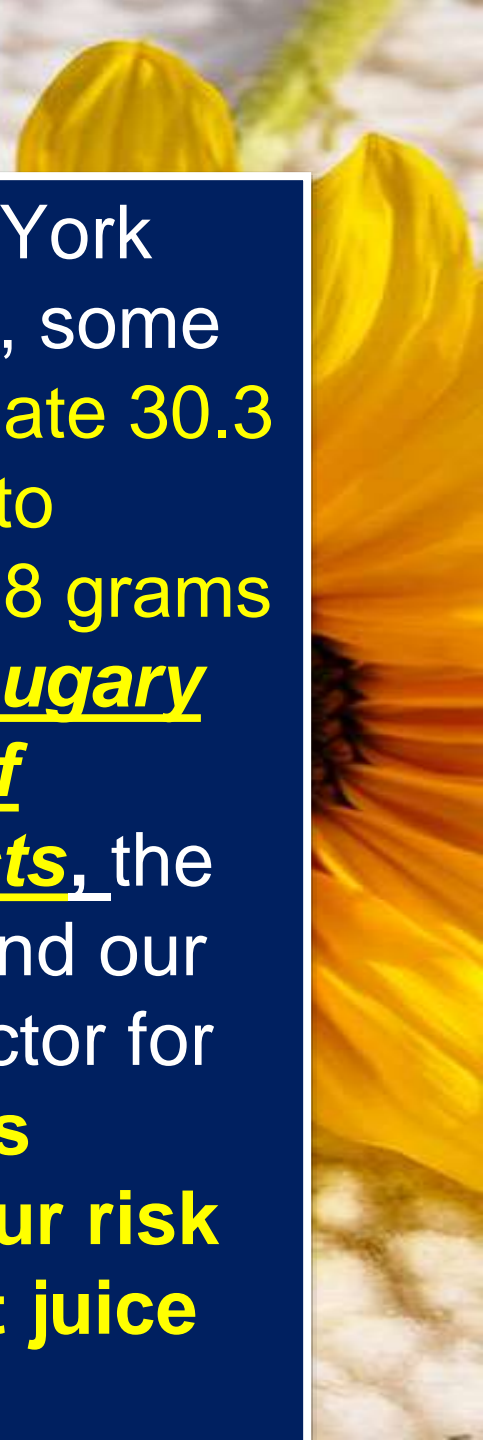
Well Played Pepsi,  
Well played...

which are  
Furthermore,  
**Mexican**  
lay an  
d the  
eating and  
**childbearing**  
**Mexican**  
**hs of life.**



# Sugar- Alzheimer's

- Researchers studied 2,226 elderly people who lived in New York City over the course of seven years. Of the total participants, some 429 developed Alzheimer's. The scientists found those who ate 30.3 grams of added sugar per day were 33 percent more likely to develop Alzheimer's compared with those who consumed 5.8 grams per day. When all categories were compared, drinking sugary soda was "significantly" associated with a higher risk of Alzheimer's when compared to other sweetened products, the authors said. "Too much sugar is linked to type 2 diabetes and our previous research has identified type 2 diabetes as a risk factor for dementia," Dr. Doug Brown said. **"This study backs up this evidence, suggesting that excess sugar may increase our risk of Alzheimer's disease, and all types of sugar—from fruit juice to lemonade—have the same impact.**







- 50% More Milk. Health
- 3.5X DHA
- Sugar - 20







- The word
- the Latin v
- -Wikipedia

Hippocrates, a  
and author of t  
Father of medi  
knife” – “do no

**SWEET DELICIOUS**

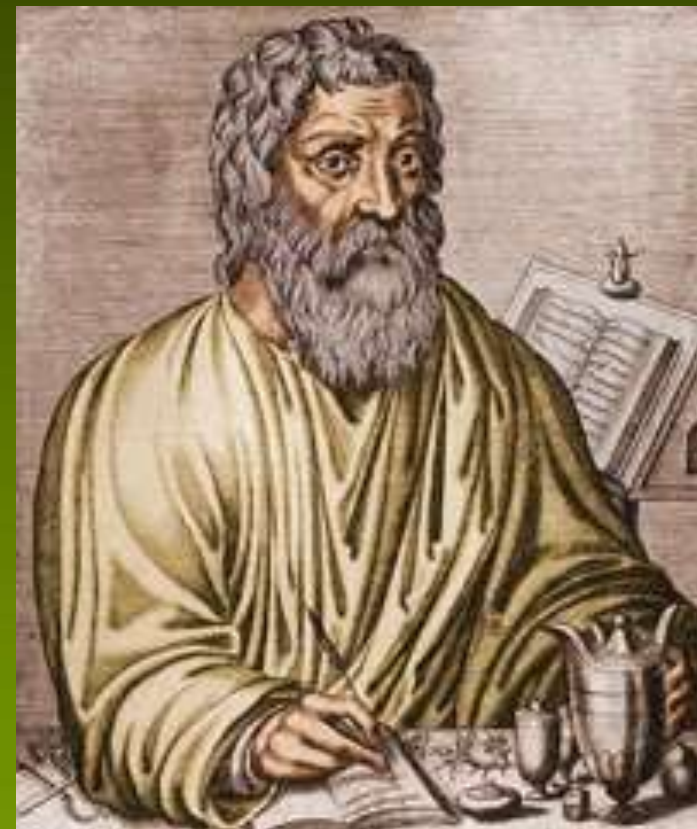
ABOUT 50% SUGAR, ABOUT 10% SUGAR, ABOUT 5% SUGAR, ABOUT 12% SUGAR, ABOUT 20% SUGAR, ABOUT 15% SUGAR, ABOUT 13% SUGAR, ABOUT 20% SUGAR, ABOUT 90% SUGAR, ABOUT 56% SUGAR, ABOUT 11% SUGAR, ABOUT 10% SUGAR, ABOUT 45% SUGAR, ABOUT 50% SUGAR, ABOUT 25% SUGAR, ABOUT 13% SUGAR, ABOUT 30% SUGAR, ABOUT 45% SUGAR, ABOUT 35% SUGAR, ABOUT 10% SUGAR, ABOUT 90% SUGAR, ABOUT 90% SUGAR, ABOUT 20% SUGAR, ABOUT 10% SUGAR, ABOUT 13% SUGAR, ABOUT 75% SUGAR

**BUT DEADLY**

Join the CAP Anti-Sugar Campaign

ns????

ntive noun of  
e] 'to teach'.





# Take Home- probiotics



- Sugar is addictive, pathogenic and very costly to ourselves and society, 20 % have diabetes, 50% have metabolic syndrome, 90% periodontal disease and cardiac.



# Probiotics and Microbiome

- **Dysbiosis**
- **Obesity**
- **NAS**
- **Cardiovascular Disease**
- **Allergies**
- **Anxiety Depression**
- **Autism Spectrum Disorder**





# Chicago- Gun violence

Causation vs correlation





Cr

# Chicago shootings, August 3–6



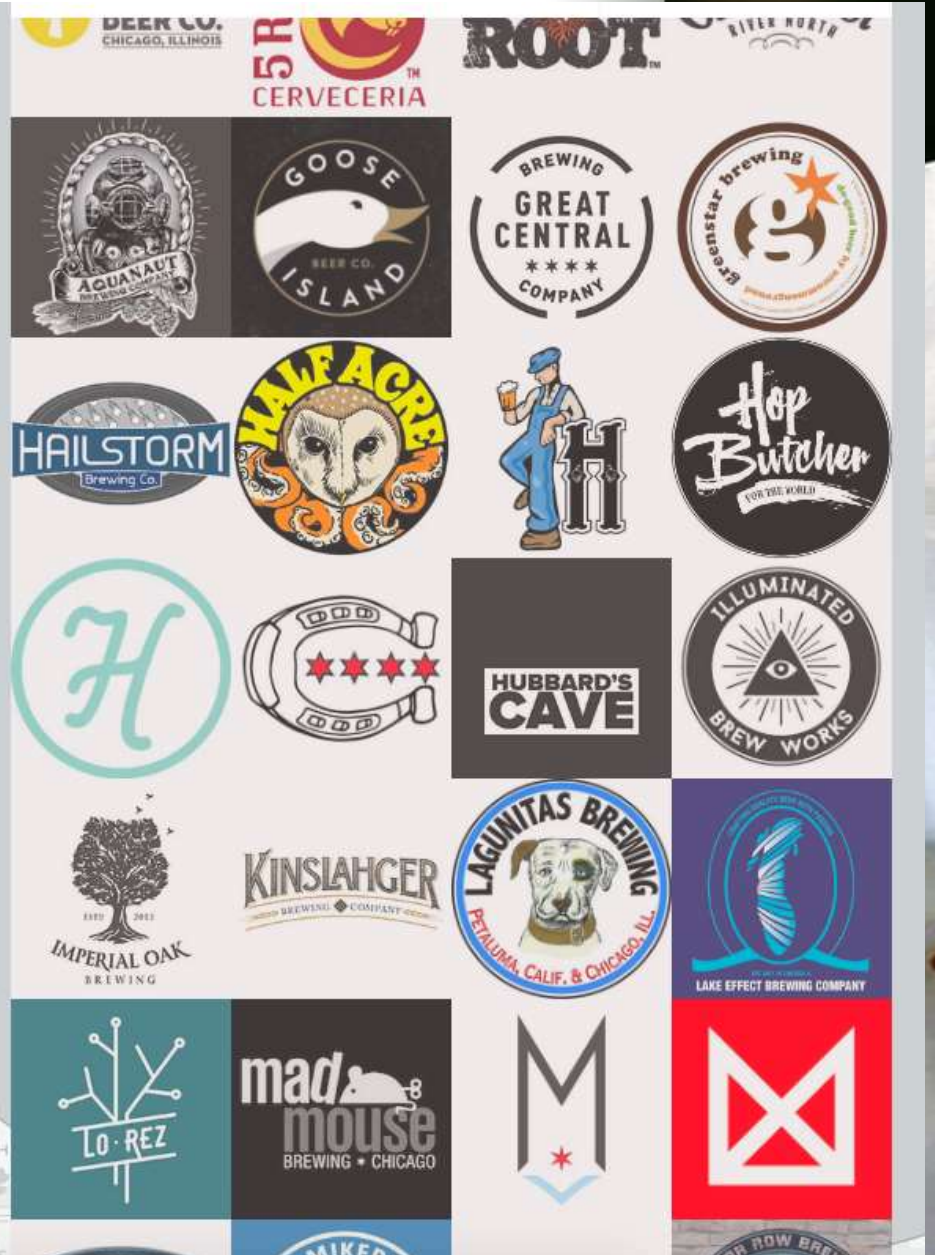
Source: Chicago Tribune

BUSINESS INSIDER





# Microbrewery- safe area





# First- Do No Harm



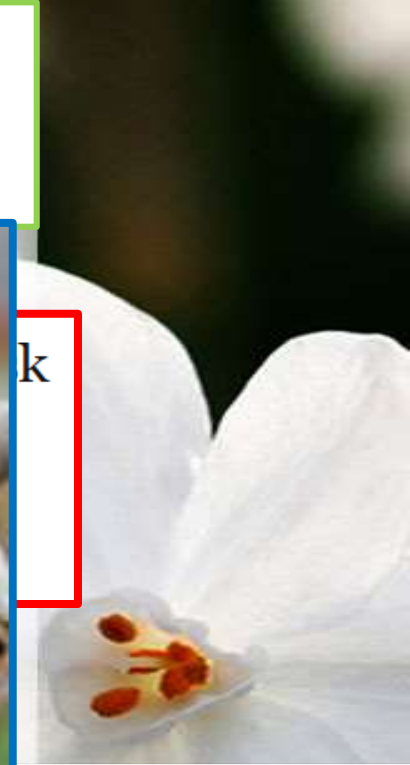
- Disinfectants cause weight gain

Postnatal exposure to household disinfectants, infant of overweight in children

Mon H. Tun, Hein M. Tun, Justin J. Mahoney, Theodore B. Konya, David S. Guttman, Allan B. Becker, Malcolm R. Sears, Jeffrey R. Brook, Wendy Lou, Tim K. Takarao, James A. Scott and Anita L. Kozyrsk  
CMAJ September 17, 2018 190 (37) E1097-E1107; DOI: <https://doi.org/10.1503/cmaj.170809>

## “Killing comm

Among 757 infants, the abundance of specific household cleaning with disinfectants and eco manner. With more frequent use of disinfectants **became more abundant**. *Lachnospiraceae* associations of the top 30th centile of household body mass index (BMI) z score ( $p = 0.02$ ) **or obesity** ( $p = 0.04$ ) at age 3. Use of eco-friendly decreased odds of overweight or obesity independently of Enterobacteriaceae abundance



d with dependent **singly mediated higher overweight** with



# Mouth rinse is GOOD for you

- Or is it?
- Can we really sterilize the mouth?
- Do we really want to?
- What exactly are we trying to do?
- What bacteria are we killing?





# Nitrate Reducing Bacteria

- But if you kill nitrate reducing bacteria? 2004 Article

## Protective effect of salivary nitrate and microbial nitrate reductase activity against caries

J. J. Doel, M. P. Hector, C. V. Amirtham, L. A. Al-Anzan, N. Benjamin, R. P. Allaker

First published: 29 September 2004 [Full publication history](#)

DOI: 10.1111/j.1600-0722.2004.00153.x [View/save citation](#)

Cited by (CrossRef): 35 articles [Check for updates](#) | [Citation tools](#) ▼



[View issue TOC](#)  
Volume 112, Issue 5  
October 2004  
Pages 424-428

**“Nitrate reducing commensals limit growth of cariogenic bacteria”**

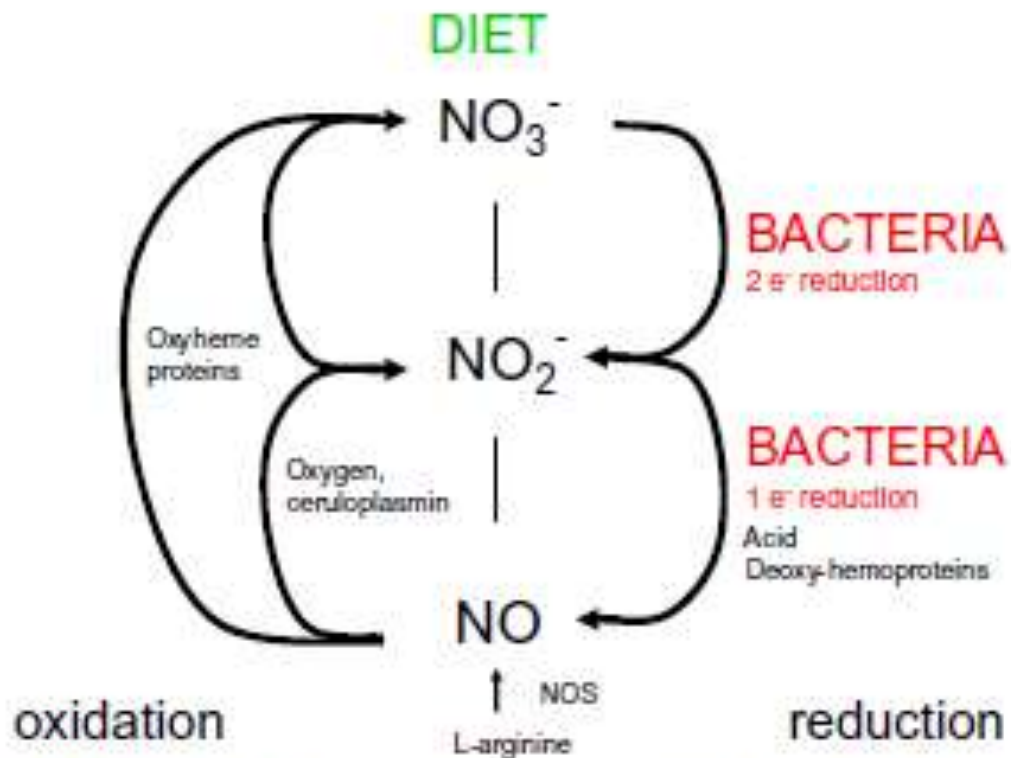
Compared with control subjects, a significant reduction in caries experience was found in patients with high salivary nitrate and high nitrate-reducing ability.

Production of nitrite from salivary nitrate by commensal nitrate-reducing bacteria may limit the growth of cariogenic bacteria as a result of the production of antimicrobial oxides of nitrogen, including nitric oxide.



## Oral Microbiome and Nitric Oxide in the Management of Blood Pressure

Nathan S. Bryan<sup>1</sup> • Gena Tribble<sup>2</sup> • Nikola Angelov<sup>2</sup>



**Fig. 1** The human nitrogen cycle whereby nitrate is serially reduced to nitrite and NO providing the host with a source of bioactive NO

In various animal models and in humans, dietary nitrate supplementation has shown numerous beneficial effects, including a reduction in blood pressure, protection against ischemia reperfusion damage, restoration of NO homeostasis with associated cardioprotection, increased vascular regeneration after chronic ischemia, and a reversal of vascular dysfunction in the elderly



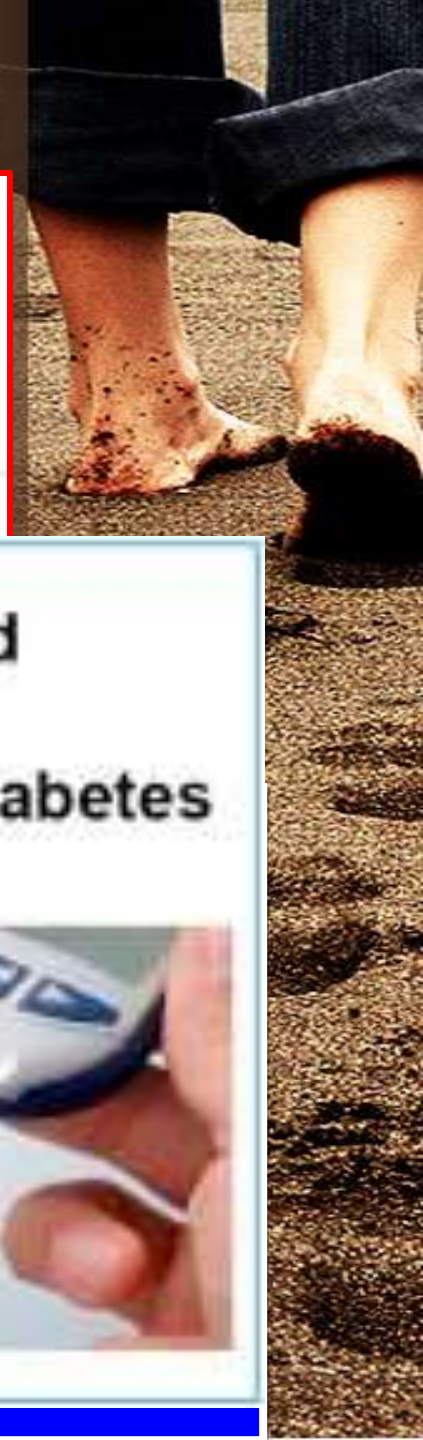
# Long Term Clinical Study



ELSEVIER

## Nitric Oxide

Volume 71, 1 December 2017, Pages 14-20



**Routine  
mouthwash  
use  $\geq$  twice/day**



***Loss of enterosalivary  
nitrate reduction  
by oral bacteria?***



**Increased  
risk for  
pre-diabetes/diabetes**





# Alcohol

Australian Dermatology Journal  
The official journal of the Australian Society of Dermatologists

Free Access

## The role of alcohol in the prevention of oral cancer: a reference to alcohol-free mouthwashes

MJ McCullough, CS Farah

- On the basis of this review, **there is insufficient evidence to accept that alcohol-free mouthwashes control the development of oral cancer** and further research is needed for dental professionals to recommend alcohol-free mouthwashes. Oral cancer is a leading cause of cancer deaths in Australia, with over 800 new cases diagnosed each year. **Despite the fact that the five-year survival rate for oral cancer is only 50%,**

Listerine	Thymol, menthol, eucalyptol, methyl salicylate	No information	Altana Pharma Ltda. imported by Johnson & Johnson Industrial Ltda. (São Paulo, Brazil).	Water; alcohol; poloxamer 407; benzoic acid; sodium benzoate; caramel coloring.
Colgate Plax Whitening	Menthol	No information	Colgate - Palmolive Indústria e Comércio Ltda. (São Paulo, Brazil).	Water, ethyl alcohol, sorbitol, poloxamer 338, polysorbate 20, sodium saccharin, CL 42090, methyl salicylate, 1.5% Hydrogen Peroxide.
Listerine Total Care	Thymol, menthol, eucalyptol, methyl salicylate	No information	Mc Neil LA LLC- Cali-Colômbia imported by Johnson & Johnson Industrial Ltda. (São Paulo, Brazil).	Water, alcohol, sorbitol, poloxamer 407; flavoring; benzoic acid, sodium benzoate, zinc chloride, sucralose, sodium saccharin, Cl 16035, Cl 42090; sodium fluoride 100 ppm F <sup>-</sup> (0.022% -).
Listerine Tartar Control	Thymol, menthol, eucalyptol, methyl salicylate	0.064% Thymol, 0.042% menthol, 0.092% eucalyptol, 0.06% methyl salicylate	Johnson & Johnson Industrial Ltda. (São Paulo, Brazil).	Purified water, 21.6% alcohol, N-propanol; sorbitol, poloxamer 407, mint flavor, benzoic acid, sodium benzoate, sodium saccharin, zinc chloride; FD & C Blue coloring No. 1.
Even Mint	Thymol, menthol, eucalyptol, methyl salicylate	No information	Indústrias Reunidas Raymundo da Fonte S.A. (Belém, Brazil)	Water, alcohol, benzoic acid; sodium benzoate; sorbitol; poloxamer 407; flavoring, allantoin, Cl 42090, Cl 19140; sodium saccharin.

ticular

**There is insufficient evidence to accept that alcohol-free mouthwashes control the development of oral cancer**

healthcare  
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as registered in  
**therapy, the  
er cent.**



# Take Home- probiotics



- Chemicals tend to kill good bacteria and not just bad pathogens. We may do more harm than good, increasing ED, Caries, Cancer and Heart disease when using mouth rinses. We need better and safer treatment.



# Prevention: 101

- **Prebiotics**

and

- **Probiotics**





# Dental Disease- Dysbiosis

- Disease, bacterial, fungal, yeast, and probably viral, are due to “**dysbiosis**”.
- Bacteria protect from, and cause dental decay, and periodontal disease.
- Esthetic dentistry may also be needed because of trauma, or genetic causes, or iatrogenic reasons

## Principles of Prevention



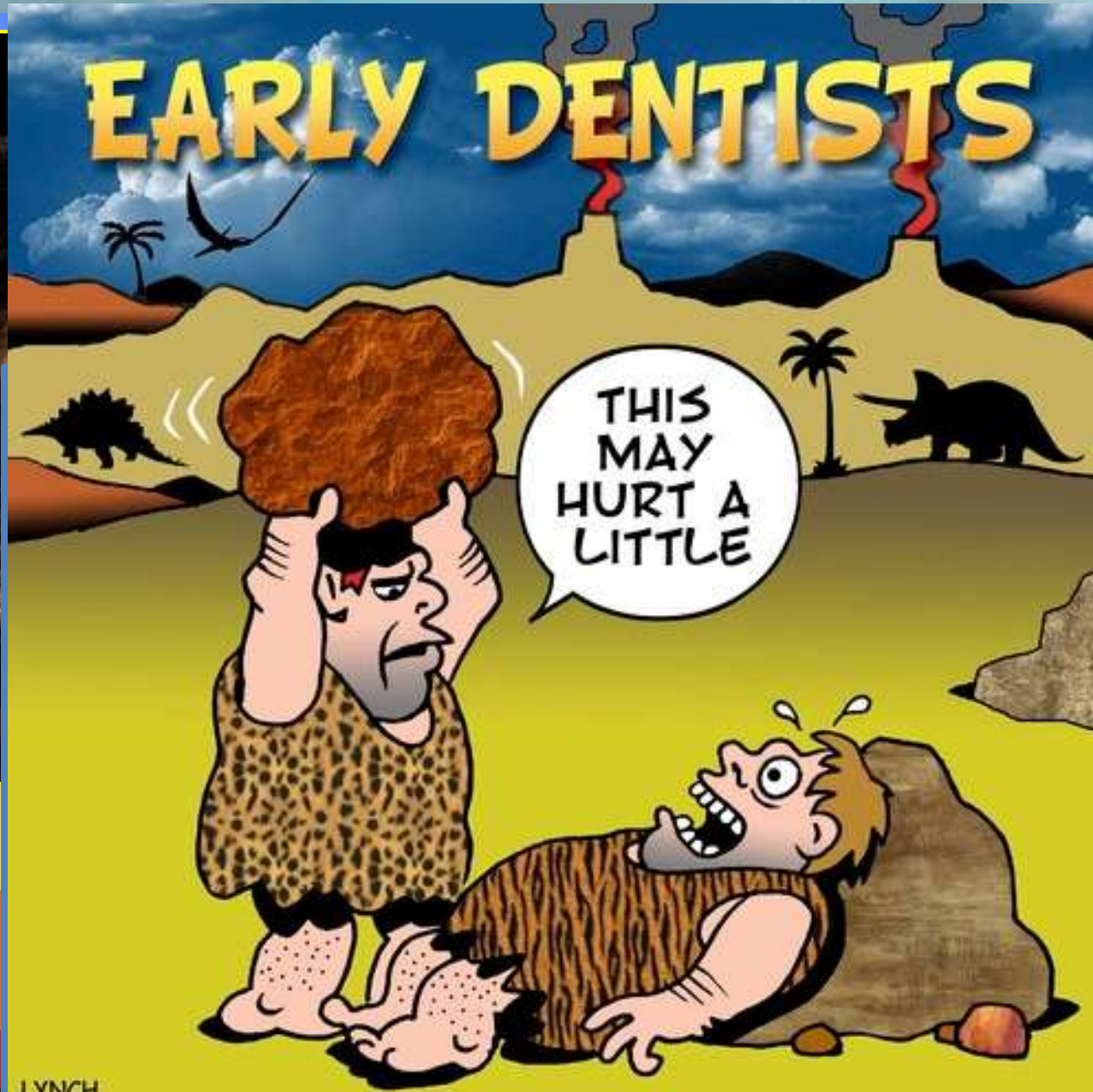


# *"Homo naledi"- Hominids*



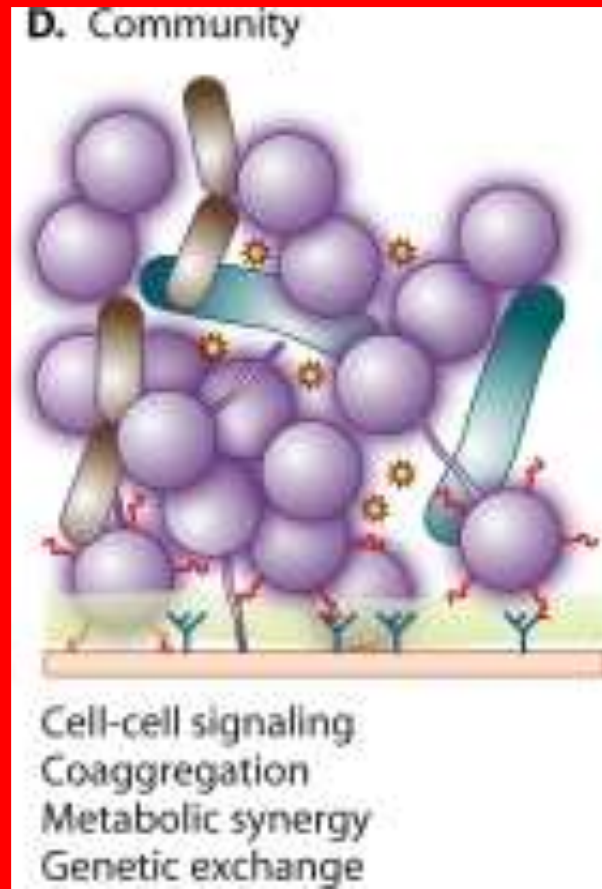
6 MILLION  
YEARS AGO

7 MILLION  
YEARS AGO





- Long range Molecules





# Prebiotics and Probiotics

- **Diagnosis:**

- **Oral**

- **CariScreen (Carifree)**
    - **CamX Spectra (Air Tech)**
    - **MyPerioPath (OralDNA Labs)**
    - **MitoSwab (MitoSwab)**
    - **GenoTek (DNA GenoTek)**





# Prebiotics and Probiotics

- Diagnosis:
  - Salivary- oral microbiome genetic info



Find Your Balance

*Ready to achieve maximum health and wellness?*

The image shows a white box for the V10ME diagnostic kit. The box is open, revealing two compartments: a dark blue one labeled 'Metabolic Intelligence' and a light beige one labeled 'Gut Intelligence'. The box is sitting on a dark blue circular base.

**1. Metabolic Intelligence**

Determines the unique way your body processes food into energy.

**2. Gut Intelligence**

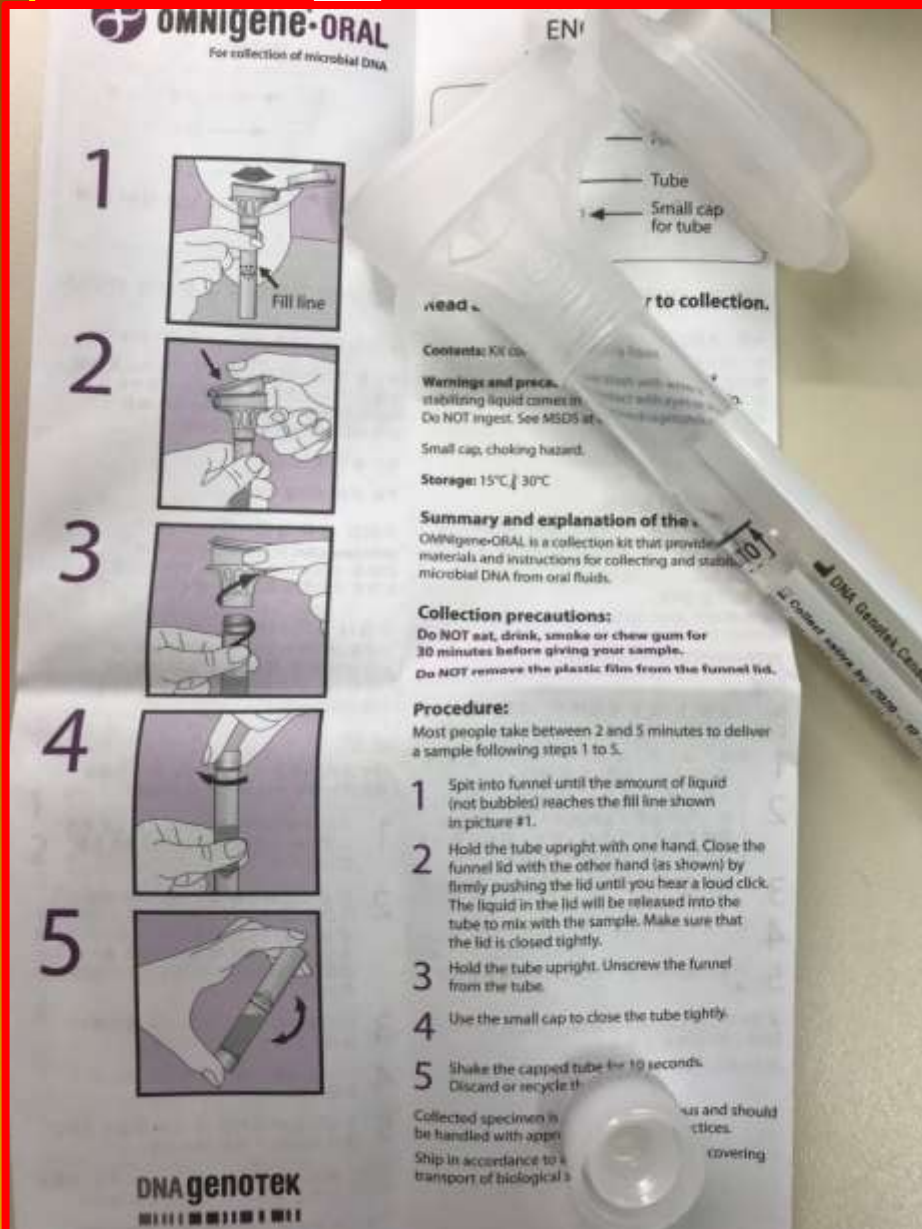
Identifies the overall health and the biochemical activity of the trillions of microbes in your gut.

V10ME





# Diagnostic tests



ek – OMNigene  
OM-501





## MITOswab Test Results

Name: Keith Cannon

Date of B

MITOswab test: - Mitochondrial respiratory chain enzyme activity are measured in patient's buccal sample.

### Result Values- (Observations)

Activity name
Total Buccal Protein yield (micrograms)
Citrate Synthase <sup>§</sup>
RC-IV (RC-IV/CS) <sup>¶</sup>
RC-I (RC-I/CS) <sup>¶</sup>

#### Notes-

§: Activity value as nanomoles/min/mg buccal protein

¶: Presented as ratio of the corresponding RC activity to

\*: Number in parenthesis indicates the percent of contr

^: Based on published data.

## MITOswab test analysis reveals -

- The overall content of mitochondria is significantly higher than - 'normal range' as indicated by the citrate synthase activity value (265% of the normal mean activity level) in test buccal sample.
- The activity of Respiratory Chain Complex-IV (RC-IV) (109% of the normal mean value) was in the normal range.
- The activity of Respiratory Chain Complex-I (RC-I) (120% of the normal mean value) was in the normal range.



Powered by

CLIA ID #: 39D2130307



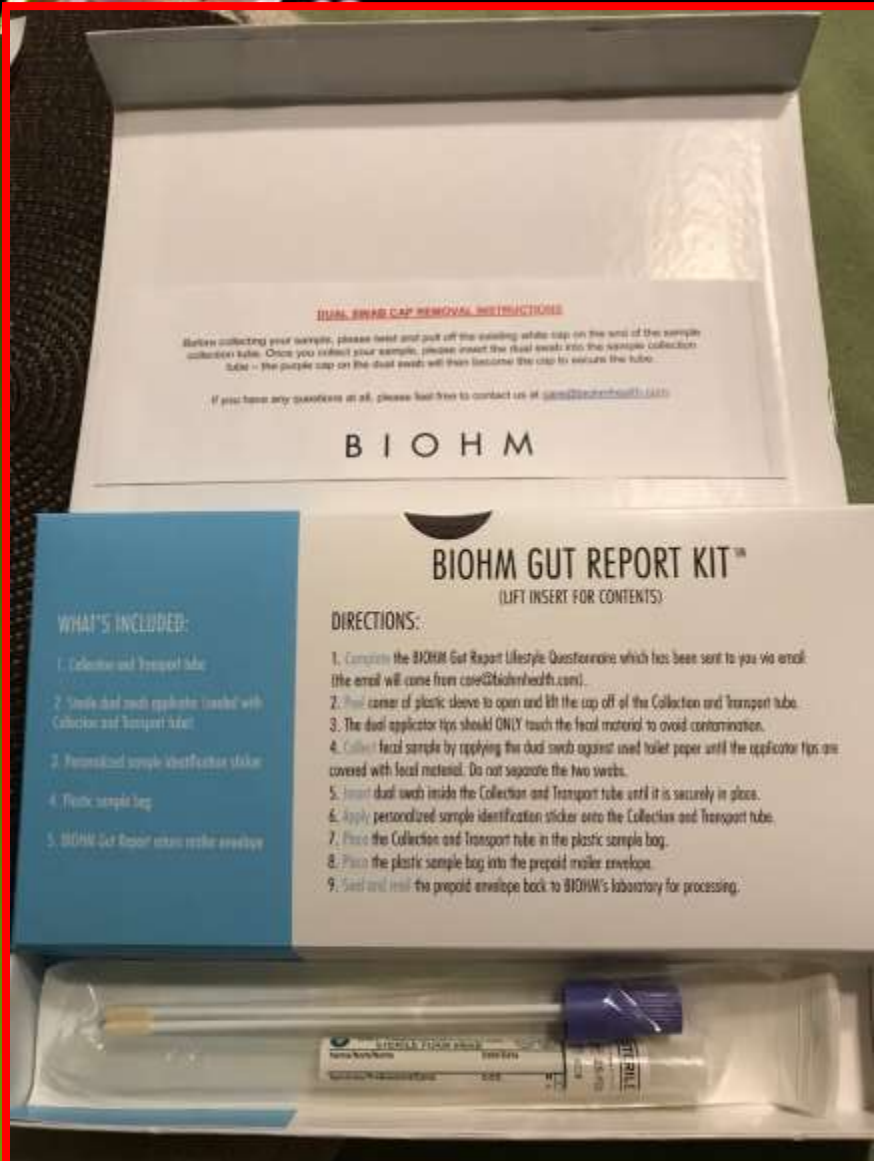
## Interpretation-

- Biochemical analysis results of subject's buccal sample suggest that it has normal range activities of RC-I and RC-IV.
- Although the RC-I and RC-IV activities are robust and in the normal range, almost 3-fold higher CS activity may suggest a compensatory or corrective response to an underlying mitochondrial dysfunction that may be present.
- Periodic mitochondrial enzyme assessment is strongly suggested, to monitor that this compensatory/corrective response is in place to keep the enzyme values in normal range.



# BIOHM Gut Report

What we need is good diagnosis! **Which probiotic to use?** How much for how long? What is the goal and what may be a side effect? Should be strain specific and tailored to your environment







# Viome- transcriptomics

Entire gut microbiome

The term can be applied to the total set of transcripts in a given organism, or to the specific subset of transcripts present in a particular cell type. Unlike the genome, which is roughly fixed for a given cell line (excluding mutations), the transcriptome can vary with external environmental conditions. Because it includes all mRNA transcripts in the cell, the transcriptome reflects the genes that are being actively expressed at any given time, with the exception of mRNA degradation phenomena such as transcriptional attenuation.



# Metabolomics



International Journal of  
*Molecular Sciences*



*Int J Mol Sci.* 2016 Jun; 17(6): 870.

Published online 2016 Jun 2. doi: [10.3390/ijms17060870](https://doi.org/10.3390/ijms17060870)

PMCID: PMC4926404

PMID: [27271597](https://pubmed.ncbi.nlm.nih.gov/27271597/)

## Metabolomic Studies of Oral Biofilm, Oral Cancer, and Beyond

[Jumpei Washio](#)\* and [Nobuhiro Takahashi](#)

- **Metabolomics** is the large-scale study of small molecules, commonly known as metabolites, within cells, biofluids, tissues or organisms. Collectively, these small molecules and their interactions within a biological system are known as the **metabolome**.





• Metam  
returne

## Metamatrix.

Clinical Laboratory  
3425 Corporate Way  
Duluth, GA 30096  
770.446.5483 Fax: 770.441.2237

Ordering Physician:  
Associated Dental Specialists  
Mark Cannon DDS, MS  
Grove Medical Center STE 308  
RDD 4160  
Long Grove, IL 60047

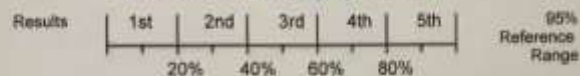
Accession Number: **A1110270144**  
Reference Number:  
Patient: **Ryan P. Cannon**  
Age: **26** Sex: **Male**  
Date of Birth: **12/30/1984**  
Date Collected: **Not Specified**  
Date Received: **10/27/11**  
Report Date: **11/11/11**  
Telephone: **1 (847) 634-6166**  
Fax: **1 (847) 634-6302**  
Reprinted:  
Comment: **FAX Results**

Date of collection not provided; specimen validity and test results are questionable.

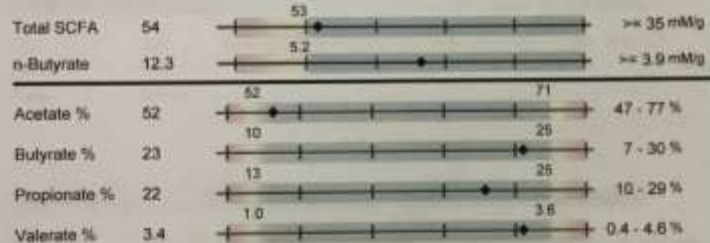
Methodology: DNA Analysis, GC/MS, Microscopic,  
Colorimetric, Automated Chemistry, ELISA

### 2100 Gastrointestinal Function Profile

#### Percentile Ranking by Quintile



#### Beneficial SCFA



#### Inflammation



#### Immunology



#### Beneficial SCFA

Short chain fatty acids (SCFA) are produced by bacterial fermentation of dietary polysaccharides and fiber. The product, N-butyrate, is taken up and used to sustain the normal activity of colonic epithelial cells. Butyrate has been shown to lower the risk of colitis and colorectal cancer. A healthy balance of GI microbes depends on production of SCFA by one species to allow the normal growth of another one in a complex cross-feeding network.

#### Inflammation

Lactoferrin, an iron-binding glycoprotein, is released in IBD but not in non-inflammatory IBS. High levels are found in Crohn's, UC or infection. WBC's are elevated in general inflammation/infection. Mucus is often visualized in acute GI inflammation.

#### Immunology

High fecal sIgA indicates immune system reactions to the presence of antigens from bacteria, yeast or other microbes. Low sIgA can result from stress or malnutrition. Anti-glutadin sIgA is a screening marker for gluten sensitivity.

Georgia Lab Lic. Code #007-007  
CLIA ID# 11D0255349

New York Clinical Lab PFI #4573  
Florida Clinical Lab Lic. #80008124  
Page 3

Laboratory Director  
J. Alexander Bailey, PhD  
Robert M. David, PhD

## Metamatrix.

Clinical Laboratory  
3425 Corporate Way  
Duluth, GA 30096  
770.446.5483 Fax: 770.441.2237

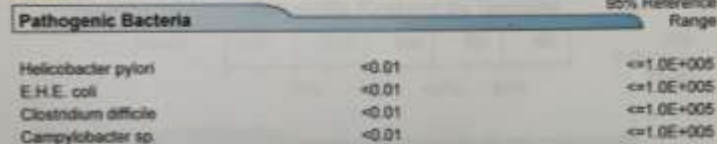
Ordering Physician:  
Associated Dental Specialists  
Mark Cannon DDS, MS  
Grove Medical Center STE 308  
RDD 4160  
Long Grove, IL 60047

Accession Number: **A1110270144**  
Reference Number:  
Patient: **Ryan P. Cannon**  
Age: **26** Sex: **Male**  
Date of Birth: **12/30/1984**  
Date Collected: **Not Specified**  
Date Received: **10/27/11**  
Report Date: **11/11/11**  
Telephone: **1 (847) 634-6166**  
Fax: **1 (847) 634-6302**  
Reprinted:  
Comment: **FAX Results**

Date of collection not provided; specimen validity and test results are questionable.

Methodology: DNA Analysis, GC/MS, Microscopic,  
Colorimetric, Automated Chemistry, ELISA

### 2100 Gastrointestinal Function Profile



#### Yeast/Fungi



#### Parasites

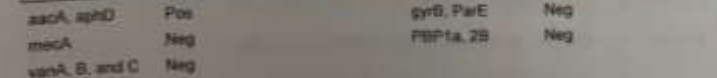


A taxonomy unavailable finding likely indicates an ingested protozoan and not a human parasite. It does not indicate treatment unless patient symptoms and other inflammatory markers are consistent with parasite infection.

#### Adiposity Index



#### Drug Resistance Genes



**Yeast/Fungi**  
Yeast overgrowth has been linked to many chronic conditions, in part because of antigenic responses in some patients to even low rates of yeast growth. Potential symptoms include diarrhea, headache, bloating, atopic dermatitis and fatigue. Positives are reported as +1, +2, +3 or +4 indicating >100, >1000, >10000 or >100000 pg DNA/g.

**Parasites**  
Parasite infections are a major cause of non-viral diarrhea. Symptoms may include constipation, gas, bloating, increased allergy response, colitis, nausea and deterioration.

The Adiposity Index is derived by using DNA probes that detect multiple genera of the phyla Firmicutes and Bacteroidetes. Abnormalities of these phyla may be associated with increased caloric extraction from food.

Georgia Lab Lic. Code #007-007  
CLIA ID# 11D0255349

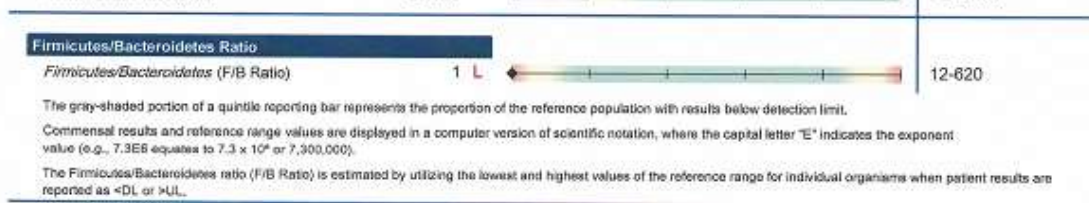
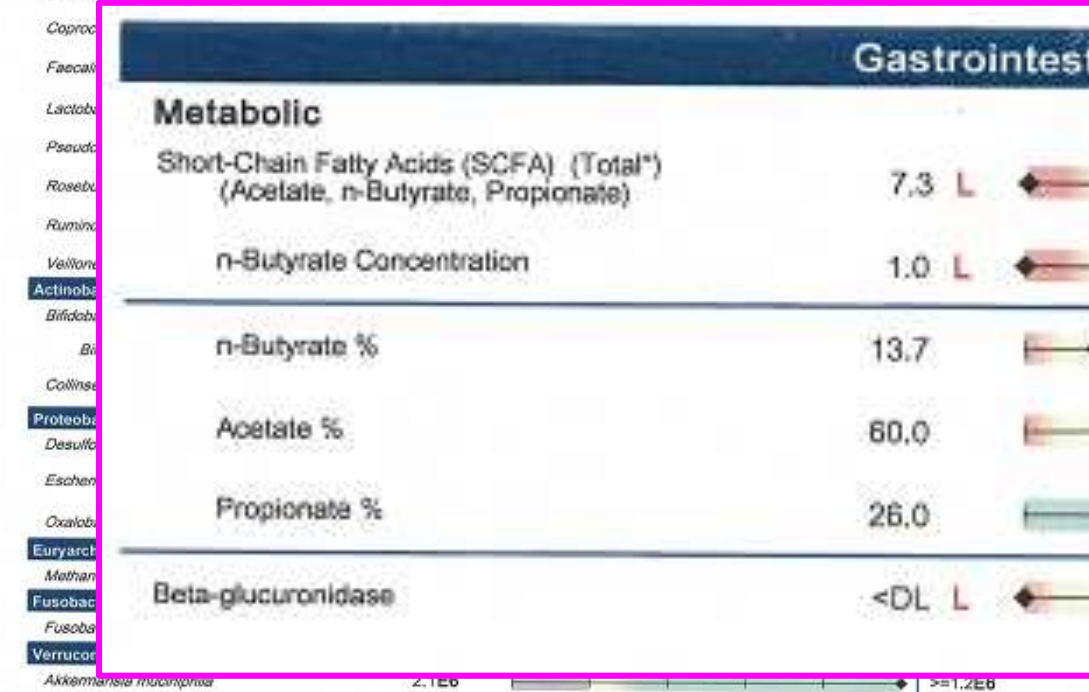
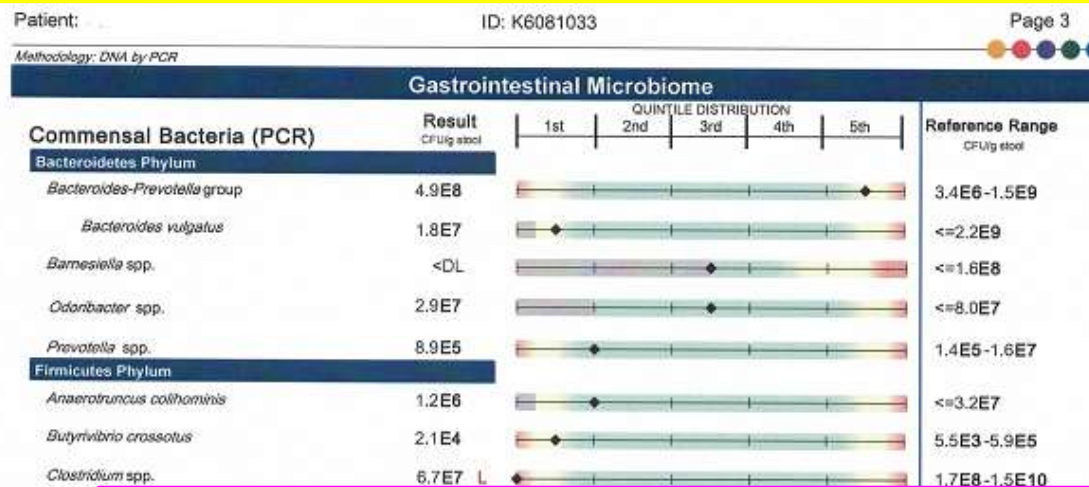
New York Clinical Lab PFI #4573  
Florida Clinical Lab Lic. #80008124  
Page 2

Laboratory Director  
J. Alexander Bailey, PhD  
Robert M. David, PhD





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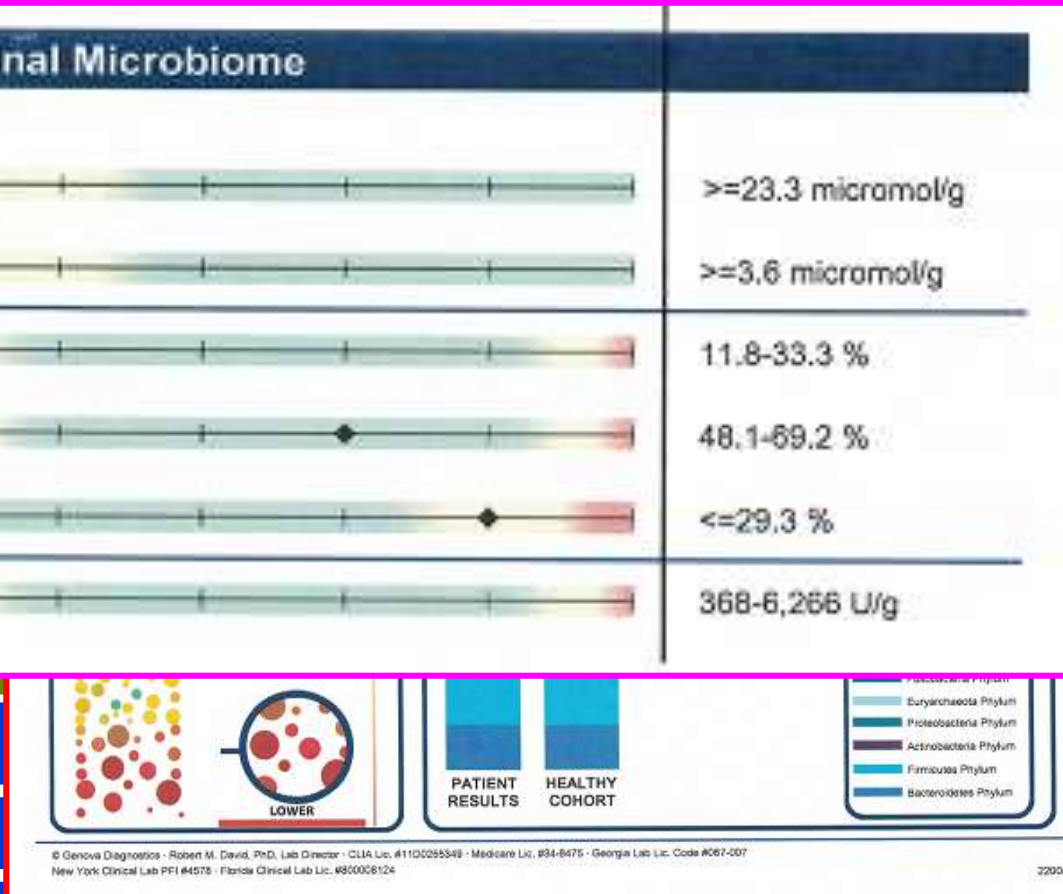


3425 Corporate Way  
Duluth, GA 30096

## GI Effects Stool Profiles

Patient: Order Number: K6081033  
DOB: Completed: March 02, 2017  
Sex: Received: February 08, 2017  
MRN: 1232796593 Collected: February 05, 2017  
Route Number: 1587960

Direct Laboratory Services  
Referring Laboratory  
4040 Florida St  
Ste 101  
Mandeville, LA 70448









## Sample, Report

Date Of Birth: 09/20/1980 (37 yrs)  
Gender: Female  
Patient ID: 851750  
Patient Location: Test Site A

## Ordering Provider

Ronald McGlennen MD  
7400 Flying Cloud Drive  
Eden Prairie, MN 55344  
855-672-5362

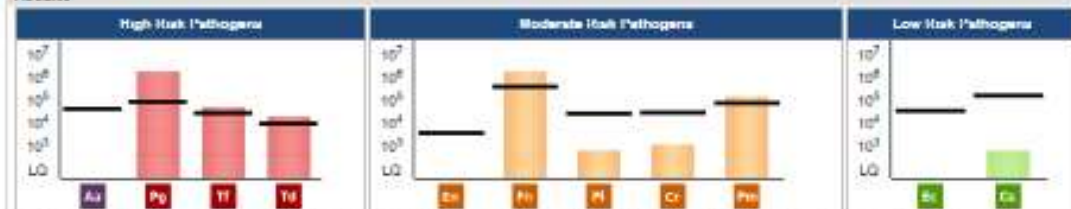
## Sample Information

Specimen#: 5033050001  
Accession#: 201807-12468  
Specimen: Oral Rinse(P)

Collected: 07/08/2018  
Received: 07/09/2018 09:57  
Reported: 07/10/2018 11:12

## MYPERIOPATH MOLECULAR ANALYSIS OF PERIODONTAL AND SYSTEMIC PATHOGENS

### Results



### Interpretation of Results

- This result shows 3 high risk (Au, Pg, Tf) and 2 moderate risk (Fi, Cr) pathogens above the therapeutic threshold.
- The bacterial species Au and Pg are strongly associated with chronic periodontitis, are transmissible and tissue invasive even at low amounts of these organisms. Moreover, Au is an anaerobic pathogen that often colonizes dental plaque, often along with other red complex bacteria. Note: the bacterial species Ec is commonly resistant to various treatments, and may be a reservoir of antibiotic resistance.
- The detected pathogens are also risk factors for various systemic diseases, including atherosclerosis, type 2 diabetes, arthritis, dementia and several types of cancer. The American Heart Association supports a causal relationship between periodontal disease and atherosclerosis. Specifically, Au has been shown to accelerate vascular disease of the aorta.

### Treatment Considerations: to be determined by the healthcare professional

- Mechanical/Debridement:** Scaling and root planing (SRP) is a mainstay of therapy to disrupt biofilm, remove plaque and debride compromised tissue. This patient harbors a series of pathogens (Au, Pg, Tf, Cr) that may be refractory to this treatment.
- Systemic Antibiotics:** This patient has indicated no allergies.

1 Clindamycin 150 or 300 mg tid for 8-10 days  
As always, use antibiotics with care.



"If your patient has a history of intolerance to the first choice consider:

- Ciprofloxacin 500 mg bid for 8-10 days
- Clarithromycin 500 mg bid for 8-10 days

- Local Antibiotics and Chemical Hygiene:** As an adjunct to SRP, sub-antimicrobial doses of doxycycline hydrate lower collagenase activity and reduce periodontal pocket depth. Alternatively, locally delivered antimicrobial agents (LDA) including minocycline microspheres, doxycycline hydrate in an absorbable polymer, or chlorhexidine in a gelatin matrix have been shown to decrease pocket depth modestly.
- Pocket or Field Decontamination:** Laser decontamination as an adjunct therapy to SRP may be beneficial in reducing probing depth and bacterial loads. The consideration of using lasers as an adjunct to SRP is dependent on type of laser used and the particular protocol.
- Chemical and Gaseous antiseptics:** Chlorhexidine or Povidone iodine rinses can reduce periodontal pocket depth. Prescription tray application of peroxide gel, as an adjunct to frequent periodontal maintenance appointments for refractory patients, demonstrated significant reductions in bleeding on probing. Ozone is a volatile antiseptic that can disrupt microbial membranes.
- Probiotics and Prebiotics:** Probiotics are live, beneficial bacteria, typically administered as a food or dietary supplement. Probiotics are non-digestible ingredients that promote growth of commensal bacteria. Research shows that prebiotics and probiotics control the growth of pathogens and reverse tissue destruction caused by periodontitis.
- Periodontal Surgery:** For severe and/or refractory periodontitis - surgical approaches such as gum flap repairs, procedures to reduce pocket depth, or other restorative procedures may be indicated.

### Follow up Recommendations

- Good periodontal health depends on compliance of a home care regimen as detailed by your healthcare provider. Daily brushing, flossing, as well as attention to nutrition, proper rest and cessation of smoking are essential.
- Follow-up testing between 8-12 weeks with MyPerioPath is recommended. Persistence of bleeding on probing is often indicative of unresolved infection. Retesting will identify residual or refractory bacteria. Currently there is not a cure for periodontal disease, only periods of remission.
- Assessment of a patient's level of inflammation with Cetus One is valuable in deciding the frequency of patient recall and treatment.

Microbiome

## Sample, Report

ID: 851750  
Date Of Birth:  
09/20/1980  
Gender: Female

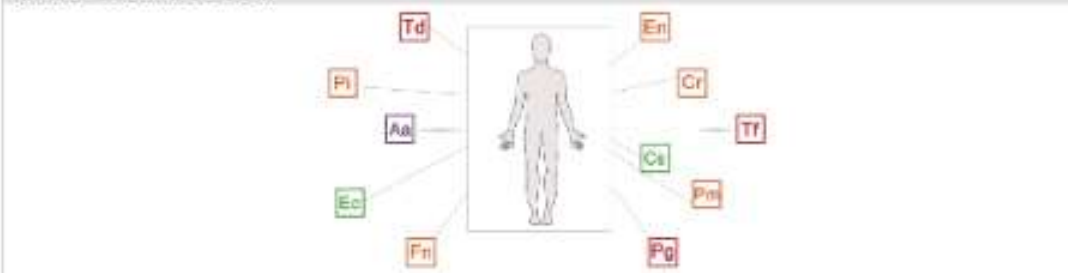
## Sample Information

Specimen#: 5033050001  
Accession#: 201807-12468  
Specimen: Oral Rinse(P)  
Collected: 07/08/2018

### Clinical Considerations

Reason for Testing	Clinical	Diagnostic	Medical History
<input checked="" type="checkbox"/> Active Periodontal Disease	<input checked="" type="checkbox"/> Redness/Discoloration <input checked="" type="checkbox"/> Inflammation/Redness <input checked="" type="checkbox"/> Bleeding on Probing	<input checked="" type="checkbox"/> Type III Moderate Periodontitis Tooth Numbers: 3 8 14 19 24 30 Pocket Depths(mm): 4 4 5 4 4 3	<input checked="" type="checkbox"/> Past History of Smoking <input checked="" type="checkbox"/> Arthritis/Auto Immune Disease

### Systemic Effects of Oral Pathogens



Cancer	Cardiovascular Health	Joint and Musculoskeletal Health	Dementia and Brain Health	Metabolic Health	Healthy Pregnancy
Chronic gum disease, involving Au, Pg, Tf, Td, Fi, Cr, Pr, Ec, En, Ca is a risk factor for the development of certain cancers including ones involving the pancreas, esophagus, colon, lungs, and the head and neck. Additionally, untreated gum disease is a cause of ongoing inflammation, which may promote the advancing growth of tumors.	Select bacteria such as Au, Pg, Tf, Td, Fi, Cr, Pr, Ec, En, Ca can leak from blood vessels in the gums and travel to the heart, where cholesterol and other lipids deposit. These bacteria can irritate inflammation in arteries, and if occluded, cause a heart attack. A goal of treatment is to minimize the levels of these bacteria as much and as long as possible.	The periodontal bacteria Au, Pg, Tf, Td, Fi, Cr, Pr, Ec, En, Ca are a cause of arthritis. The oral inflammation caused by these bacteria also leads to total body inflammation which, combined with changes in a person's immunity, may result in chronic joint diseases like rheumatoid arthritis.	Recent medical studies point to poor oral health, and high levels of the bacteria Au, Pg, Tf, Td, Fi, Cr, Pr, Ec, En, Ca in our gums, increasing the risk of developing dementia such as Alzheimer's.	Obesity, lack of exercise and chronic gum disease involving the bacteria Au, Pg, Tf, Td, Fi, Cr, Pr, Ec, En, Ca cause chronic inflammation. Inflammation can damage the pancreas where insulin is produced, possibly leading to diabetes. Also, diabetes worsens oral health by increasing the level of harmful bacteria in the gums.	Bacteria associated with gum disease, especially Au, Pg, Tf, Td, Fi, Cr, Pr, Ec, En, Ca, are known to put a pregnancy at risk for pre-term birth, decreased birth weight and even blood infection in the placenta or newborn. Every pregnant woman should be tested for these harmful bacteria.

**Methodology:** Genomic DNA is extracted from the submitted sample and tested for 10 species-specific bacteria (Au: Aggregatibacter actinomycetemcomitans, Pg: Porphyromonas gingivalis, Tf: Tannerella forsythia, Td: Treponema denticola, Cr: Fusobacterium nucleatum/periodontum, Fi: Prevotella intermedia, Pr: Capnocytophaga species (gingivalis, ochracea, putrificans) known to cause periodontal disease. The bacteria are assayed by real-time quantitative polymerase chain reaction (qPCR). Bacterial levels are reported in log 10 copies per mL of sample (e.g. 1x10<sup>3</sup> = 1000 bacteria copies per mL of collection). Cross-reactivity is possible with Lactobacillus buccalis, Fusobacterium nucleatum, Capnocytophaga granulosus and Capnocytophaga leadbetteri. This test was developed, and its performance characteristics determined by OralDNA Labs pursuant to CLIA requirements. This test has not been cleared or approved by the U.S. Food and Drug Administration. The FDA has determined that such clearance or approval is not necessary.

Ronald McGlennen MD, FCAP, FACMG, ABMG

Medical Director



# Alert 2™

## MyPerioPath® + My

Combines the most widely used test for oral pathogens that cause gum disease with inherited genetic risk to create a personalized treatment plan. All from the same simple oral rinse collection.

Thousands of healthcare professionals rely on this OralDNA® Labs test to provide:

- **Early warning of oral pathogens**
- **Personalization of therapy and treatment**
- **Identification of related systemic health risks**
- **Establish inherited genetic risk**

MYPERIOD®

### FINAL REPORT

#### Sample Report

Date Of Birth: 08/20/1970(47 yrs)  
Gender: Female  
Patient Id: 789  
Patient Location: Test Location A

#### Ordering Provider

Ronald McGlennen MD  
7400 Flying Cloud Drive  
Eden Prairie, MN 55344  
855-672-5362

#### Sample Information

Specimen#: 5033032170  
Accession#: 201807-12481  
Specimen: Oral Rinse(P)

Collected: 07/17/2018  
Received: 07/18/2018 10:28  
Reported: 07/19/2018 14:00

**Reason for Testing:** Evaluation of Systemic Disease

**Related Info:** Not Provided

**Patient History:** Not Provided

### MOLECULAR DETECTION OF IL-6 PERIODONTAL RISK FACTOR

Genotype	Risk
G/G	HIGH

#### Interpretation:

This individual's interleukin 6 genotype (IL6) is G/G. This MyPerioID result indicates your patient has a high risk for periodontal inflammation due to the genetic variation examined in this test.

#### Comments:

- **Significance:** The prevalence of the G/G genotype is reported to be higher in individuals with moderate to severe chronic periodontitis and aggressive periodontitis than in individuals with no periodontal disease. This finding was independent of other risk factors such as age, smoking, ethnic origin. The 'G' allele is associated with overproduction of interleukin-6 (IL-6) cytokine in the presence of pathogenic periodontal bacteria.

- **Risk:** Individuals carrying an IL6 G allele are associated with increased odds of the concomitant detection of *A. actinomycetemcomitans*, *P. gingivalis* and *T. forsythensis*.

- **Consider:** IL-6 is a potent stimulator of osteoclast differentiation and bone resorption, is an inhibitor of bone formation, and overproduction has been implicated in systemic diseases such as juvenile chronic arthritis, rheumatoid arthritis, osteoporosis, Paget's disease and Sjogren's syndrome. The MyPerioID test assesses one of several risk factors that should be included in an overall evaluation of periodontal disease. Specific bacteria are associated with the initiation of the periodontal disease. Additional risk factors including other genetic markers, smoking, diabetes, and oral hygiene have an amplifying effect on disease progression and duration. The incidence of IL6 genotypes is reported to vary by ethnicity. Additional testing, such as MyPerioPath, may be considered if not already performed.

**Methodology:** Genomic DNA is extracted and tested for the interleukin 6 genetic variation located at position -174 (rs1800795). This genetic variation is tested by methods of the polymerase chain reaction, endonuclease digestion and resultant restriction fragment detection by automated microcapillary electrophoresis.

**Disclaimer:** The reported genotypes are a subset of the group of genes that comprises the complete immune system. This genetic analysis may not detect specific immunologic diseases or predict the health and effectiveness of a person's immunity for specific diseases. Such an evaluation may require genetic counseling and testing directed to characterize these genetic conditions. This test was developed and its performance characteristics determined by OralDNA Labs. It has not been cleared or approved by the US Food and Drug Administration.

*Ronald C. McGlennen*

Ronald McGlennen MD, FCAP, FACMG, ABMG  
Medical Director



# OralDNA Labs- Quest Diagnostics Company

**U.S. based clinical laboratory- went to Nashville- ( many years ago!) met with Dr. Tom Nabors**



- DNA(bacterial) Testing (**MyPerioPath<sup>sm</sup>**) establishes bacterial risk and can help guide therapy based on causation
- DNA (genetic) Testing (**MyPerioID<sup>sm</sup> PST®**) establishes genetic risk and can help guide therapy based on genetics
- DNA (viral) Testing (**OraRiskHPV**) identifies HPV status (separate risk factor for oral cancers)



OR  
Innov

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Oral and

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MYORALPATH

Just change the  
oral environment!!

LDNA LABS

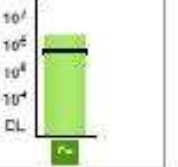
Oral and Salivary Diagnostics  
Oral Diagnostics Company

Received: 06/27/2009 07:08  
Accepted: 06/30/2009 21:45  
Revised: 07/07/2009 08:22

Am Pg Fv P Cu

Specific bacteria. Threshold  
used risk for attachment, loss,  
and other risk factors.

Low Risk Pathogens



pathogenic at relatively low

pathogenic at relatively low

pathogens; often seen in refractory

seen in refractory disease.

ed in association with other

combination with other suspected

of active disease.

s of disease; often seen in

actinobacterium nodatum

The bacteria are isolated by

5 - 100% bacteria isolate per

all with a preponderance of patients

it results due to poor sampling

It has not been isolated or

www.oraldna.com  
PMS Page 1 of 5



# Age One Visit Test

Age One

Recommen

- Streptococcus*
- Scardovia*
- Slackia ex*
- Nocardia*
- Streptococcus*
- Actinomyces*

DNA

©





# Oral Saliva Tests

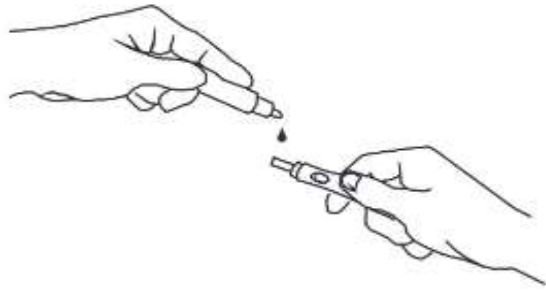


## *H. pylori* Saliva Test

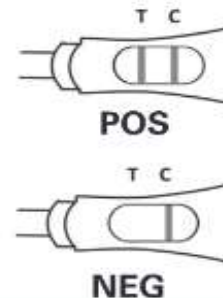


**1** Place the stick in the patient's mouth for 2 minutes

**2** Remove the stick from the patient's mouth and add two drops of buffer solution on the stick



**3** Interpret test results after 5 minutes



The *H. pylori* saliva test is a one step immunochromatographic assay for the rapid detection of *H. pylori* antigen in human saliva.

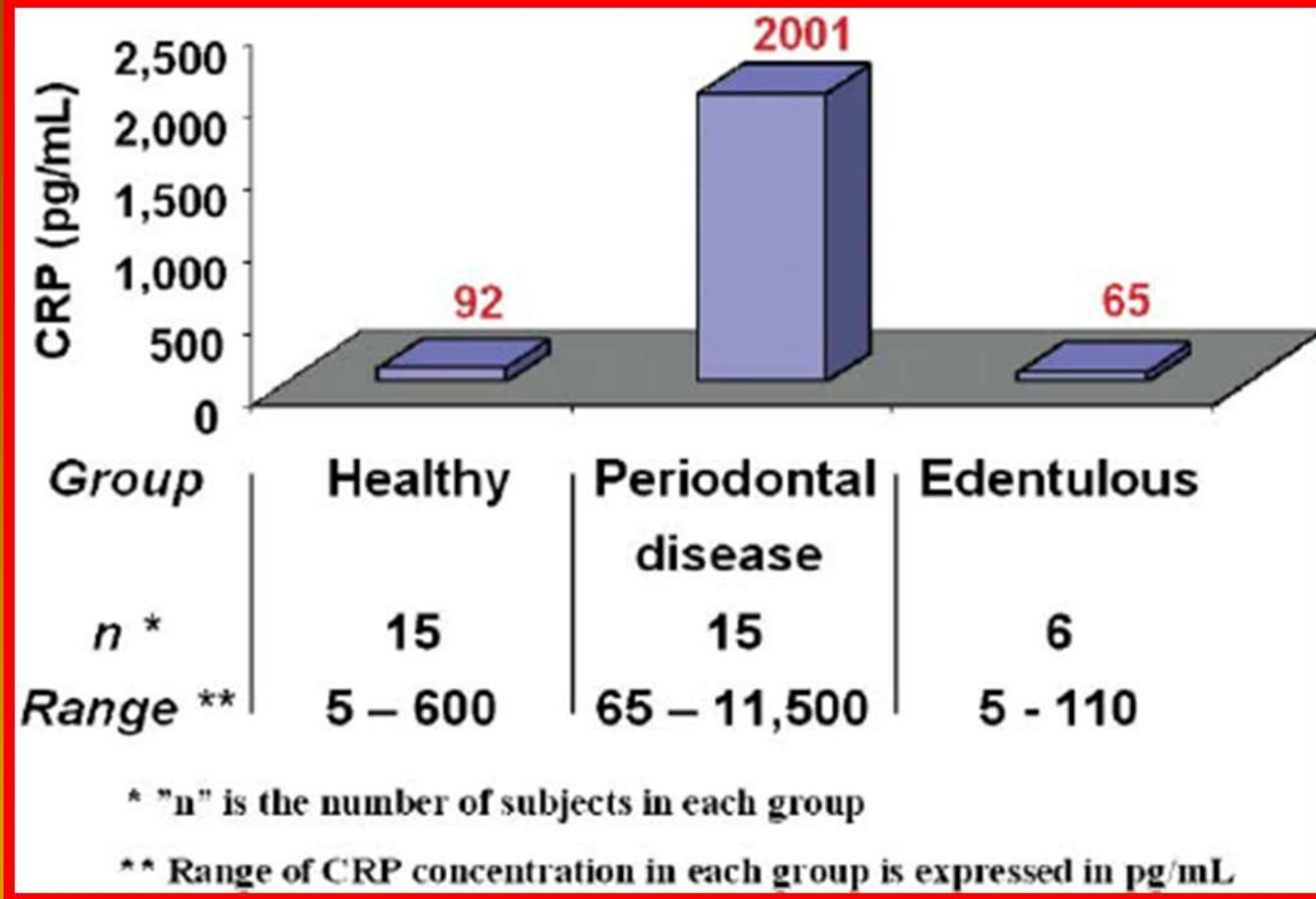


# Application of microchip assay system for the measurement of C-reactive protein in human saliva

Nicolaos Christodoulides,<sup>a</sup> Sanghamitra Mohanty,<sup>a</sup> Craig S. Miller,<sup>d</sup> M. Chris Langub,<sup>e</sup> Pierre N. Floriano,<sup>a</sup> Priya Dharshan,<sup>a</sup> Mehnaaz F. Ali,<sup>a</sup> Bruce Bernard,<sup>a</sup> Dwight Romanovicz,<sup>a</sup> Eric Anslyn,<sup>a</sup> Philip C. Fox<sup>f</sup> and John T. McDevitt<sup>\*abc</sup>

Received 4th October 2004, Accepted 9th December 2004

First published as an Advance Article on the web 13th January 2005



14 years ago-  
and then what?



# What do I use?

## Next-Generation Sequencing for Studying the Human Microbiome

The advent of next-generation sequencing (NGS) enabled several high-profile collaborative projects including the [Human Microbiome Project](#) and [MetaHIT](#), which have published a wide range of data on the human microbiome using NGS as a foundational tool.

The throughput and cost savings of NGS have fueled metagenomics studies capable of surveying the genomes of entire communities, including those of unculturable organisms.

Experimental NGS methods for analyzing the human microbiome include:

### Shotgun metagenomic sequencing

- A DNA sequencing method that enables comprehensive sampling of all genes in all organisms in a given complex microbial sample.

### 16S rRNA sequencing

- A 16S ribosomal RNA gene sequencing method used to identify and compare bacteria present within a given sample.

### Microbial metatranscriptomics

- Analysis of all RNAs encoded by a group of microorganisms within a complex sample.

only- not patient treatment (sic) Center for Genetic Medicine



# Shows up before clinical signs

Journal of Indian Society of Periodontology

Wolters Kluwer -- Medknow Publications

Identical mitochondrial somatic mutations unique to chronic periodontitis and coronary artery disease

Tokala Pallavi, Rampalli Viswa Chandra, [...], and Anumala Naveen

- Among the complete mtDNA sequences, a total of 162 variations were spread across the whole mitochondrial genome and *present only in the coronary artery and the gingival tissue samples* but not in the blood samples. Among the 162 variations, 12 were novel and four of the 12 novel variations were found in mitochondrial NADH dehydrogenase subunit 5 complex I gene (33.3%).



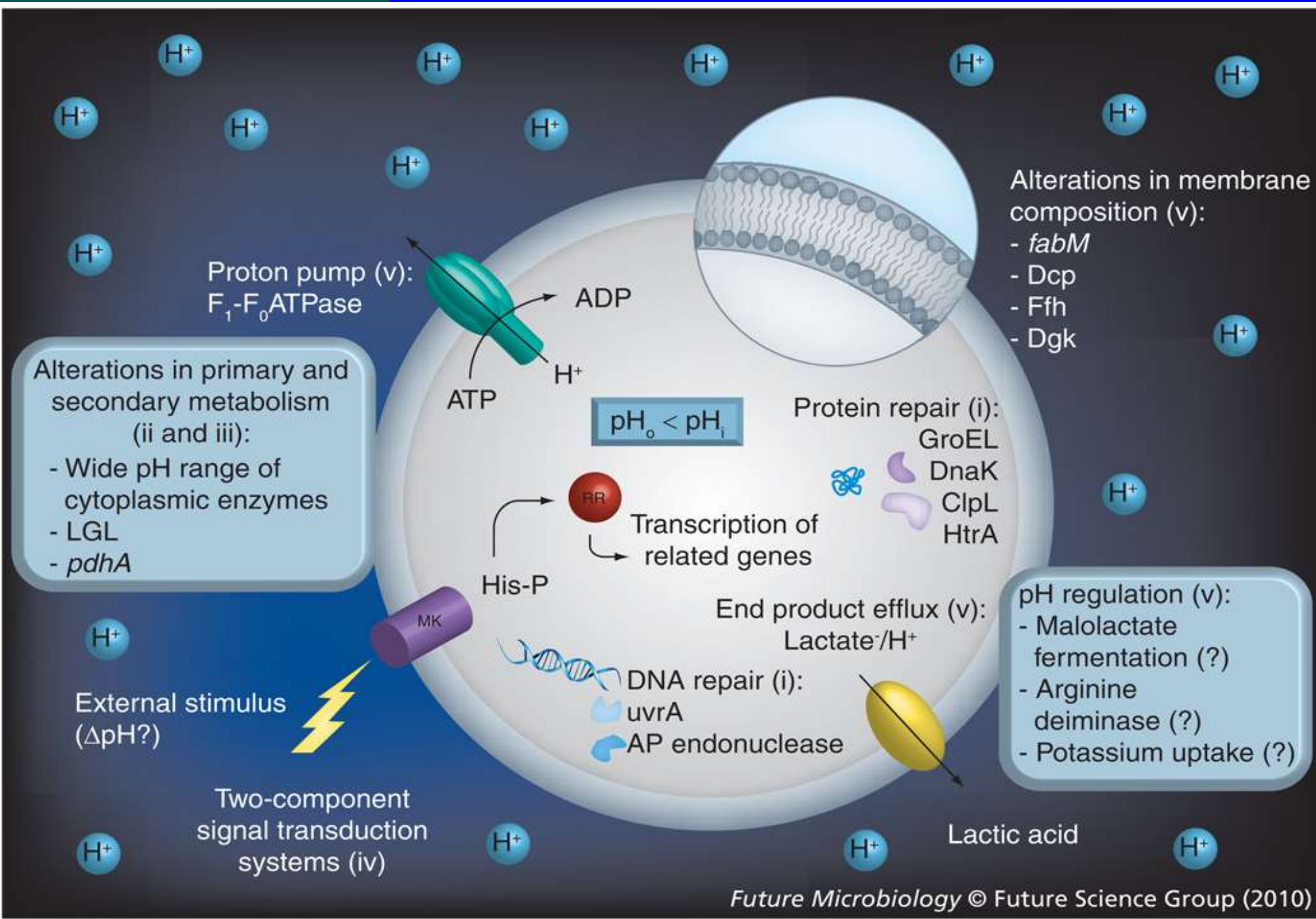


# Take Home- probiotics



- Testing is important! Microbiome testing can be accomplished with American Gut, Genova, BIOHM, Viome, uBiome and from Oral DNA labs.
- Many more tests will be available in the future.





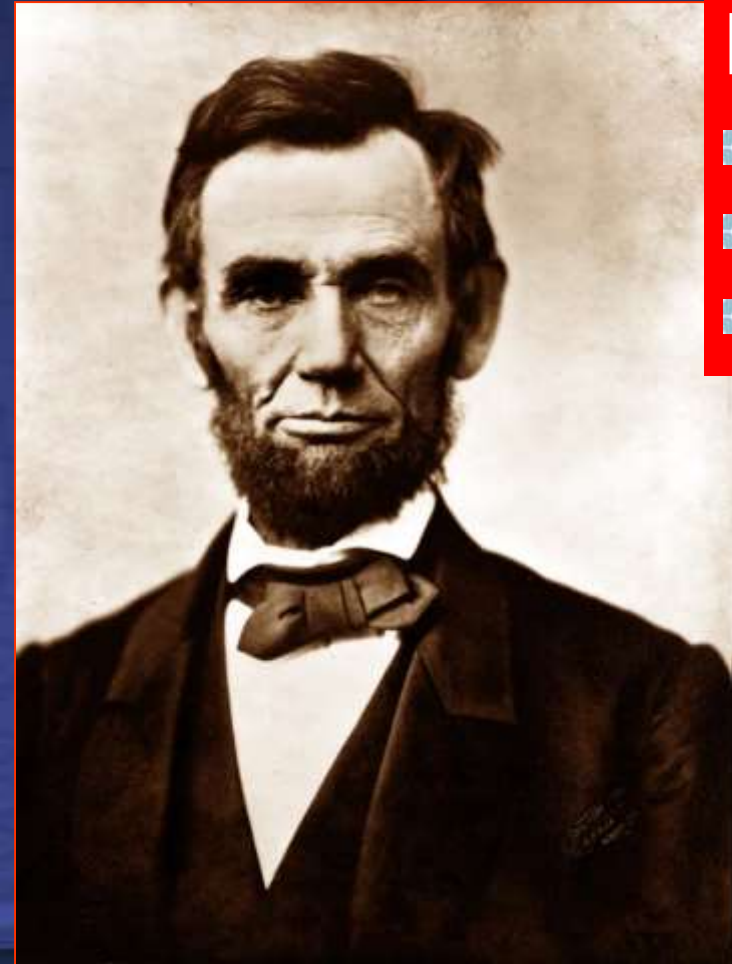
# **OCCUS**

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# Caries Risk Assessment

## Interpreting the Results



### Risk Indication Values (RLU's)

- 0-1500 = low risk
- 1501-3500 = moderate risk
- 3501-9999 = high risk



**“And in the end it's not the years in your life that count. It's the life in your years.”**



# Caries Risk Assessment

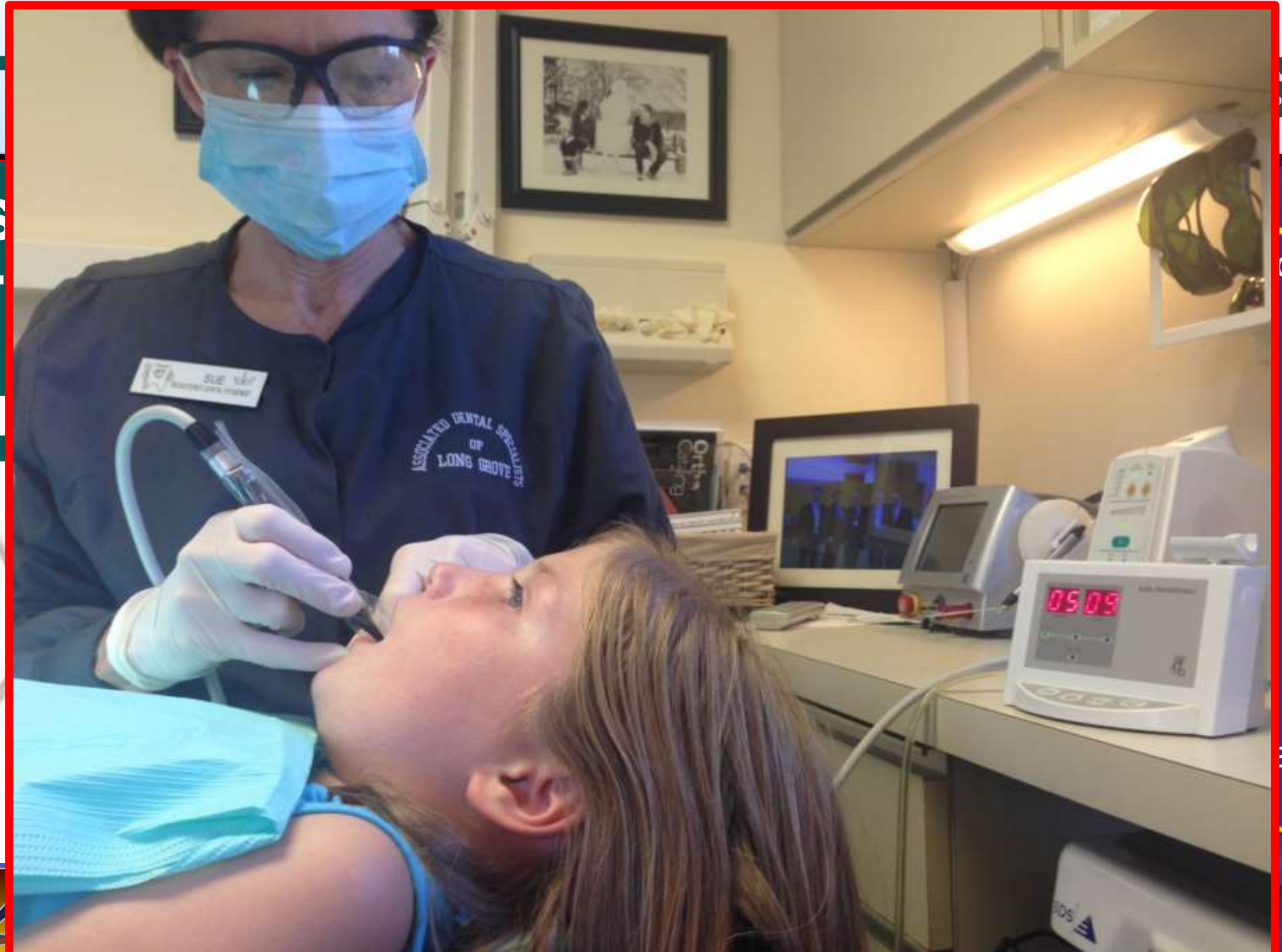
## Interpreting the Results





# Pediatric

- Caries Risk Test (CRT)
- Diagnoder



Pediatric Radiology



# What's New?! CamX Spectra



62<sup>nd</sup>  
RCA Congress  
Brussels, Belgium  
July 1-4, 2015

The 62nd Congress of the  
European Organisation for Caries Research

caries produce red fluorescence and is a fingerprint of early lesion formation. Has an advantage over the pinpoint caries detection method (DiagnoDENT).



analyzed with regard to the intensity ratio of the red porphyrin fluorescence and the green autofluorescence of the enamel. Wherever the red/green-ratio was statistically significantly higher ( $2\sigma$ ) than the red to green ratio of sound enamel a lesion was assigned. Three different methods were used to calculate the fluorescence activity of the lesions: Firstly, the maximum of the red to green ratio in a lesion, secondly the integrated red to green ratio over the lesion area, thirdly the lesion area. These findings were compared with the results of histological sectioning (gold standard). It was found that the red/green-ratio correlates with the results of the gold standard, e.g. the specificity of the first method is near 80%. Compared to pointlike fluorescence probes the camera has the advantage that the promising results concerning caries detection are less dependent on the positioning of the tip which may avoid time consuming search and alignment procedures.



# Research on CamX Spectra

J Clin Dent. 2012;23(1):1-6.

## **In vitro evaluation of the Spectra early caries detection system.**

Grave M<sup>1</sup>, Markowitz K, Strickland M, Guzy G, Burke M, Houpt M.

### **⊕ Author information**

### **Abstract**

Light induced fluorescence of tooth material- compared to clinical, radiographic and histological assessments

**CONCLUSION:** Spectra images illustrate the full spectrum of caries severity, from enamel demineralization to dentin decay. The Spectra is a promising technology for the diagnosis and for monitoring the progression of occlusal caries.

**RESULTS:** Teeth lacking radiographic caries had a mean Spectra reading of 1.5. Teeth having radiographic caries had a mean Spectra reading of 2.0. This difference was statistically significant. In general, higher ICDAS scores were associated with higher Spectra readings. Teeth with histologically evident deep dentin caries had significantly higher Spectra readings than intact teeth or teeth with superficial enamel demineralization. Spectra assessment of occlusal caries agrees with clinical and radiographic methods.

***Pediatric Oral Care***



# CamX Spectra- Sealants and Operative

## Clinical Case

- 7 year old male patient presents for pit and fissure sealants and restoration for second primary molar
- PSP radiograph obtained on patient that has severe gag reflex and **CamX Spectra scanned- nitrous oxide analgesia used and STA local anesthesia**



*Pediatric Oral Care*





Don't Have  
a Cow!  
I Just Bit  
My Tongue.





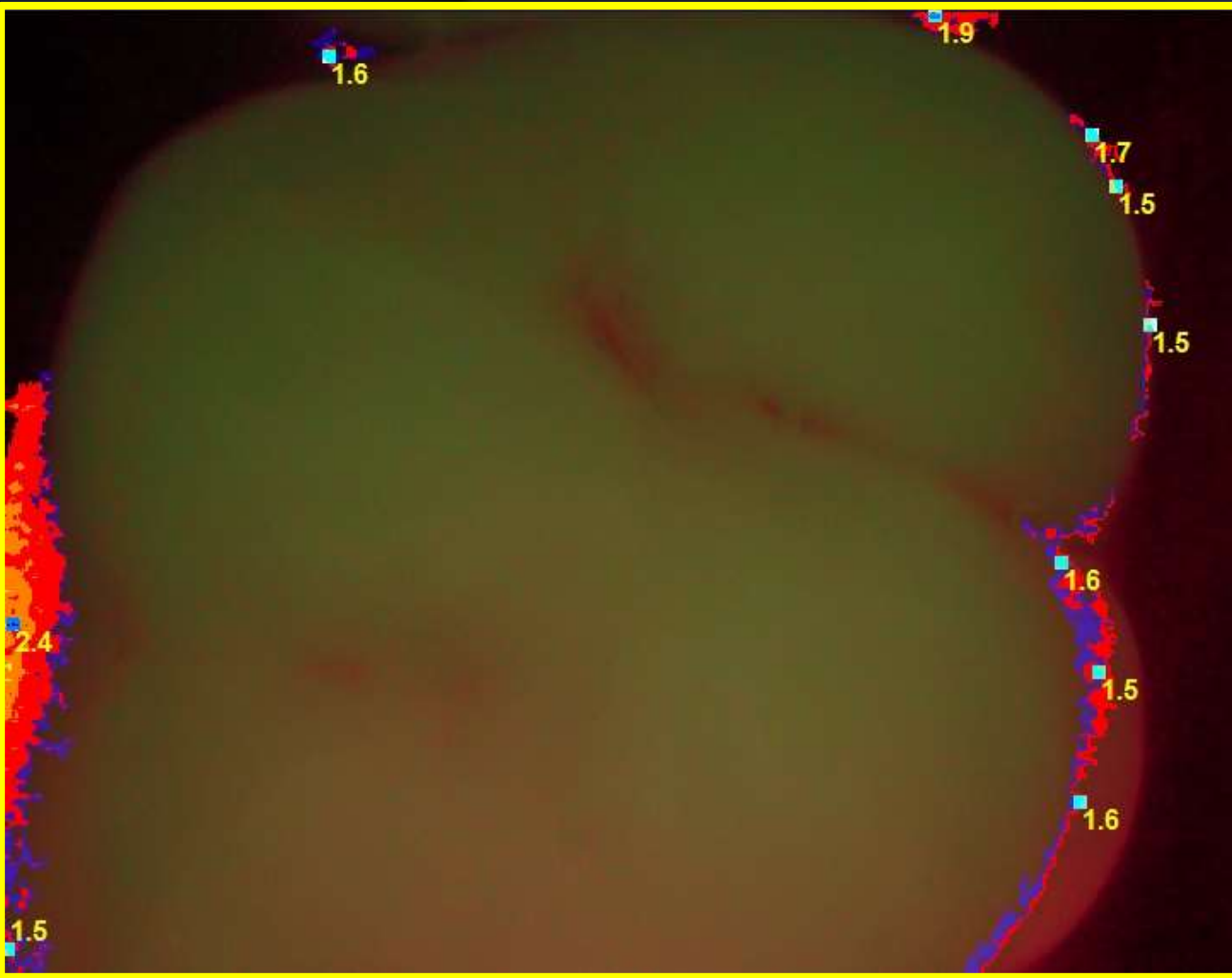
# Spectra- CAMBRA and Prevention

Spectra  
image as  
seen by  
patient  
showing  
bacterial  
fluorescence





# Spectra- CAMBRA and Prevention



Molar that was  
treatment planned for  
restoration by another  
dentist

Now a **“watch”**  
Started on a **re-  
mineralization  
protocol**

*Pediatric Oral Care*



# Scale of 0-5, now have the CDT Codes: D0601,D0602, D0603



**CamX Spectra**  
scan of first  
permanent molars  
prior to sealing to  
insure decay free  
before procedure!  
**PERIO Evaluation?**

*Pediatric Oral Care*



# ProlacSan and FotoSan Therapy



FotoSan® Blue agent  
Liquid 0.5 ml.

FotoSan® Blue agent  
Gel 0.5 ml.

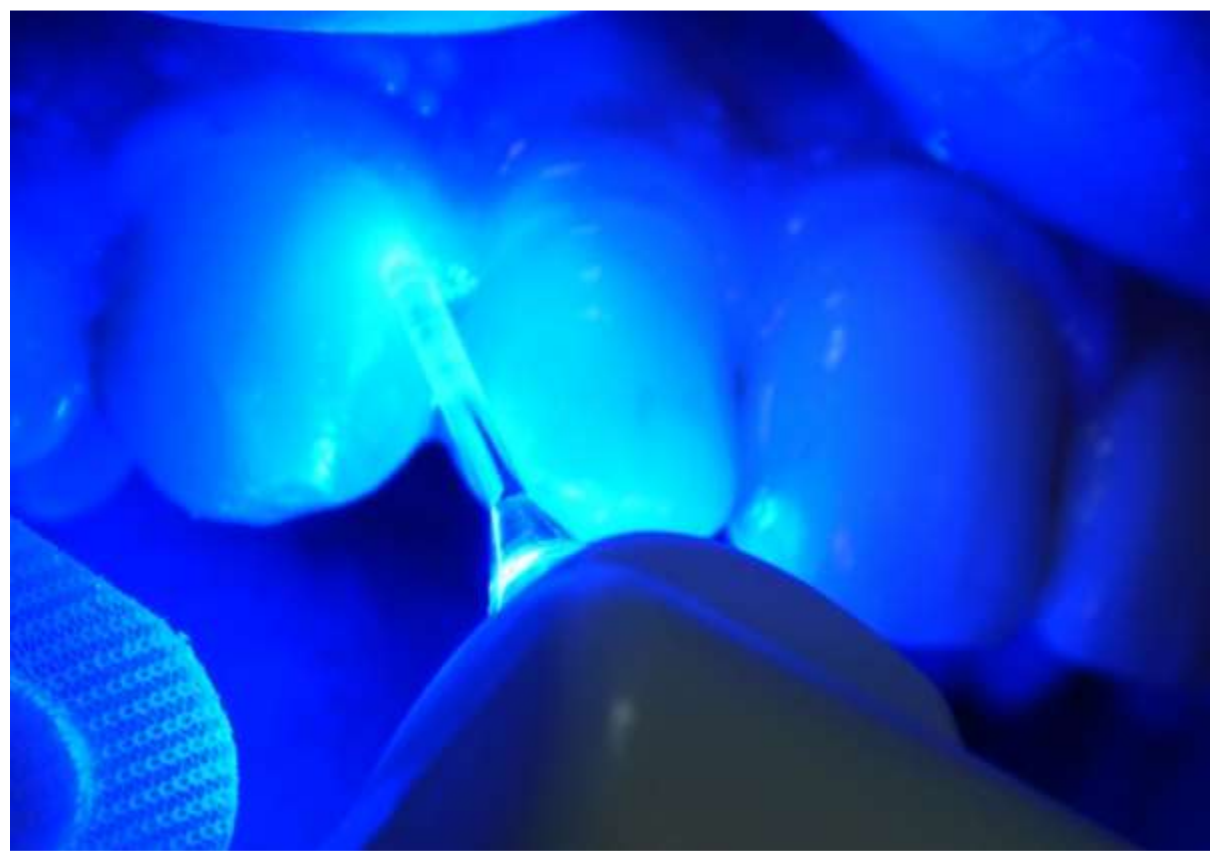
FotoSan® Blue agent  
Gel 1.5 ml.





# ProlacSan and FotoSan Therapy

- FotoSan
  - Light Activated Disinfection





# ProlacSan and FotoSan Therapy

- ProlacSan
  - *Lactobacilli brevis and plantarum*, provided as gel and as tablets



## Subgingival

### Bacterial replacement therapy

Boost the bacterial shift to healthy ones by injecting the ProlacSan® Gel directly into all the treated pockets.

The gel contains *Lactobacillus brevis* and *plantarum*.

The chosen species have excellent abilities to aggregate and adhere to mucosa and tooth surfaces. This means that the probiotic species do not flush out of the pocket as a chemical would.

## ProlacSan®

30 lozenges. Each tablet contains  $1.2 \times 10^{10}$  probiotics, a mix of *Lactobacillus brevis* and *plantarum*. Mint taste. Let the tablet melt in the mouth. Shelf-life 24 months.





# Take Home- probiotics



- Testing done with Cariscreen, OralDNA, CamX Spectra, and treating with Replacement Therapy.



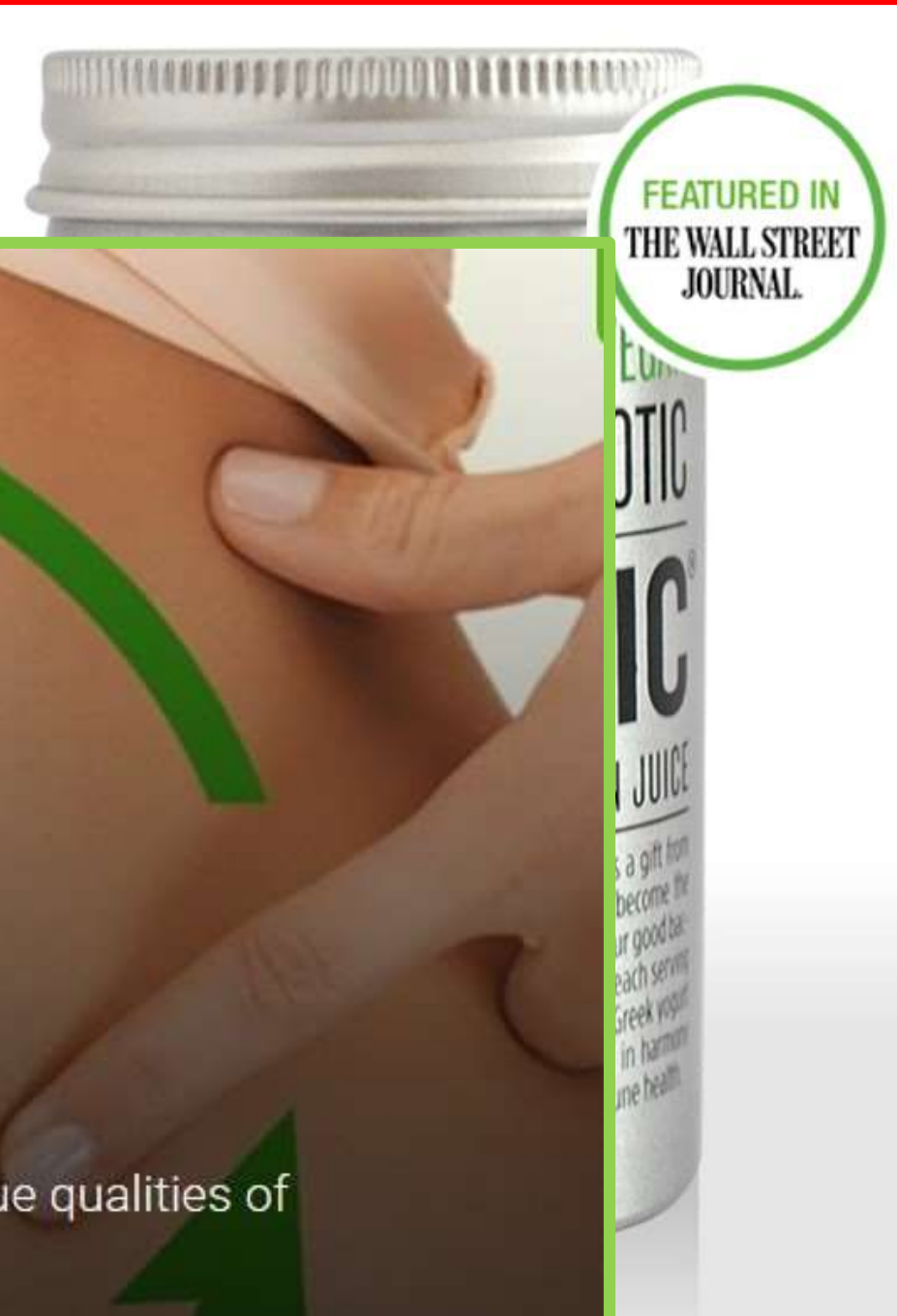


# **Probiotics- Replacing Nature**

**How did this all begin?**



Gary B. Huffnagle, Ph.D., is Professor of Internal Medicine, Microbiology, and Immunology, University of Michigan Medical Center. His research on probiotics has appeared in leading scientific journals and has been featured in *Newsweek*, *Forbes*, and on *BBC News*.



## ABOUT PROBIOTICS

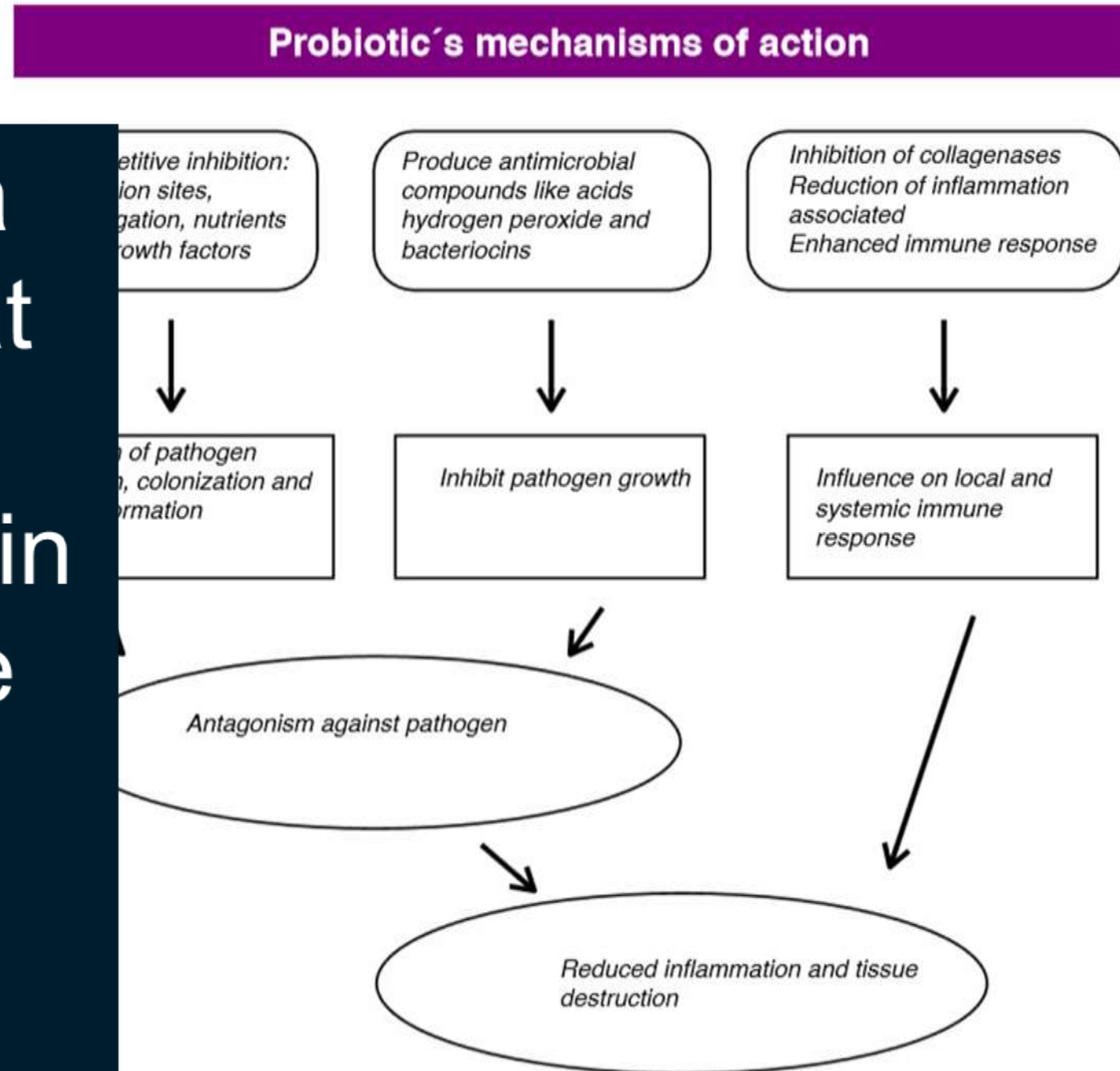
Each probiotic has different characteristics depending on the unique qualities of its good bacteria and the medium in which they are grown.

*Lactobacillus*



# Probiotics

Pathogen= bacteria  
in the wrong place at  
the wrong time  
Probiotic= bacteria in  
the right place at the  
right time  
- Dr. Cannon's  
Definition





# Probiotics – great interest in research

The number of published studies investigating probiotics has increased 15 fold in as many years, and 2011 looks set to be a record year with more than 1300 publications anticipated.

An analysis of publications posted to the NIH PubMed database reveals strong growth in the number of publications featuring the keyword 'probiotic'. In 1997 there were roughly 80 publications globally per year referencing probiotics, today that figure is over 1200 per year or 100 publications per month.

## Preservation of Antibiotics for Medical Treatment Act H.R. 1549/S. 619

▣2015: Over 1400

▣Many new Journals







# Probiotics

ted formula on IgA

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2004;20:22-6.

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15. Kosloske AM. Epidemiology of necrotizing enterocolitis.  
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16. Szajewska H, Setty M, Mrukowicz J, Guandalini S. Probi  
Gastrointestinal Diseases in Children: Hard and Not-So-Hard  
Evidence of Efficacy. *J Pediatr Gastroenterol Nutr* 2006;42(6):  
75.



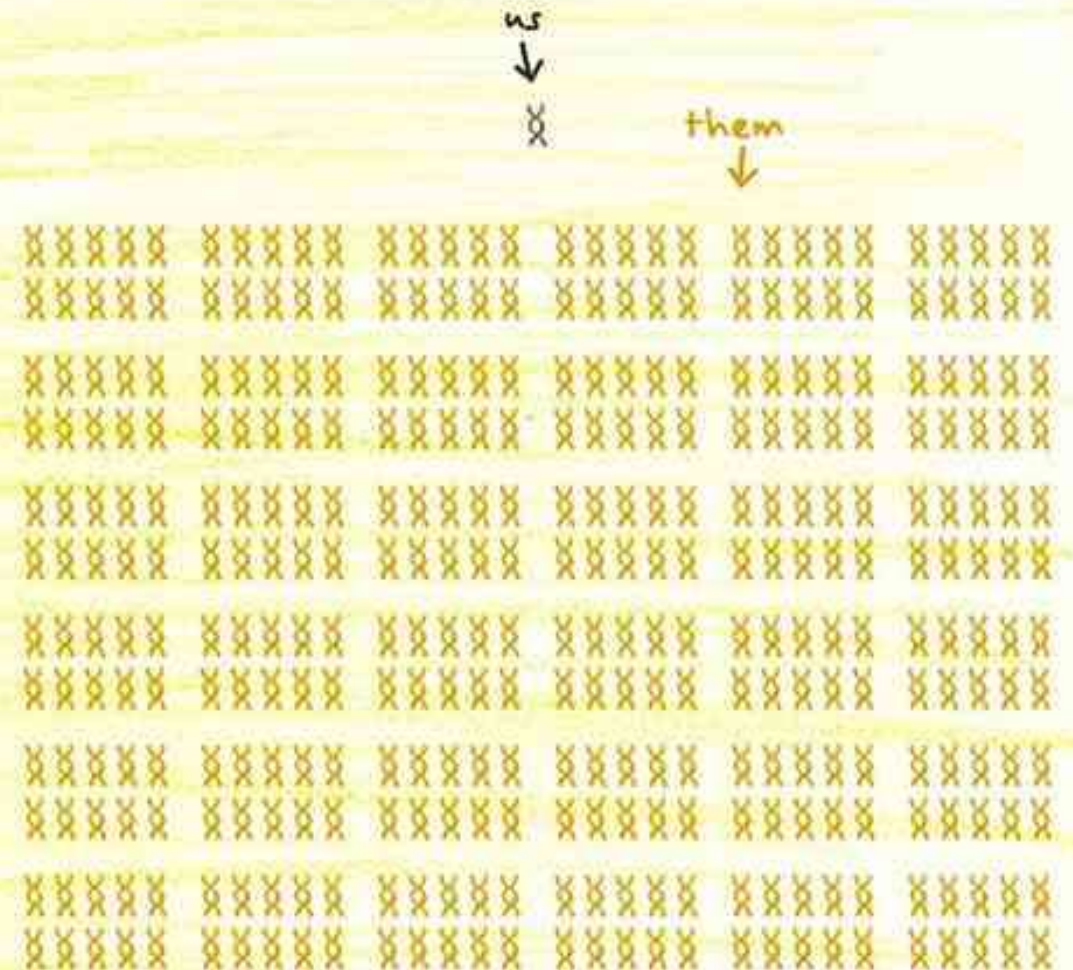
- Modulation  
improve



# MATERNAL IMPRINTING “Mom Knows Best”

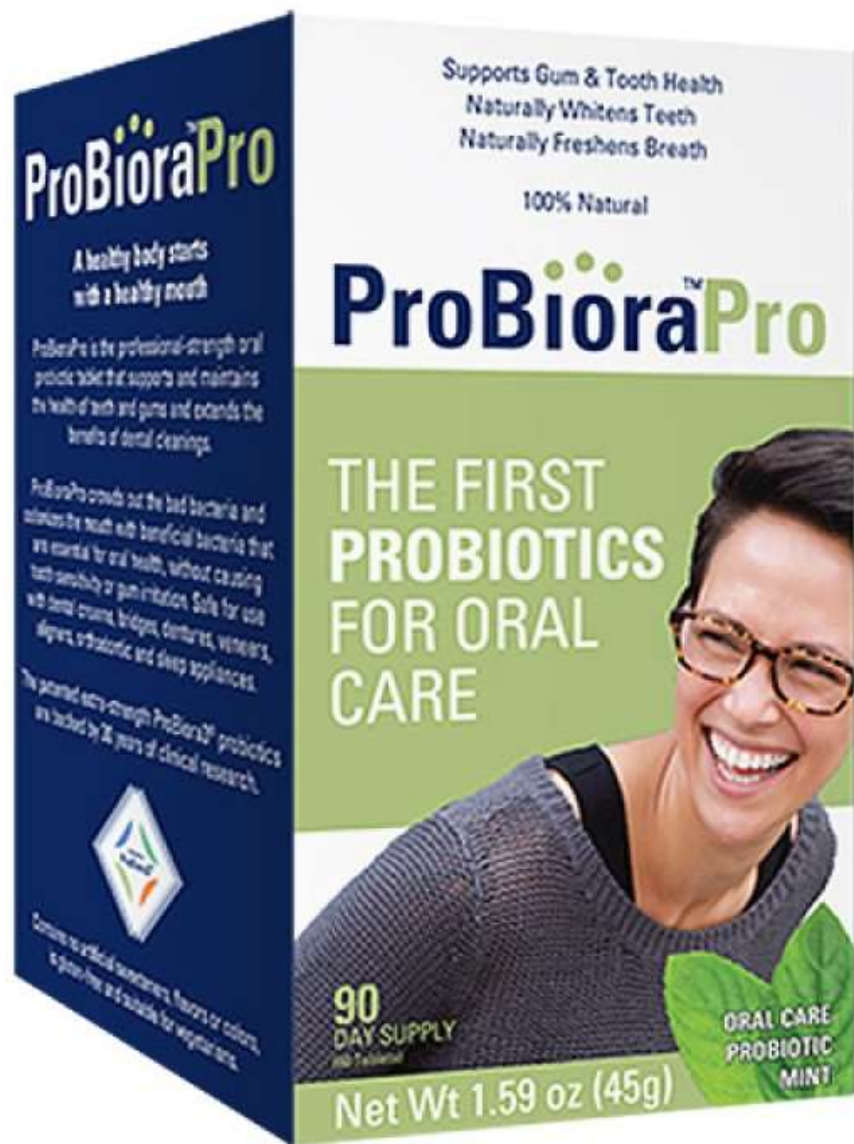
CONCE  
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molec  
pathog

For every HUMAN gene in your body, there are 360 microbial genes.





Material  
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# Lactobacilli

Species	Human	Pig	Chicken	Cattle
<i>L. acidophilus</i> Group				
<i>L. acidophilus</i> (A-1) <sup>b</sup>	?			
<i>L. amylovorus</i> (A-3)		M	?	?
<i>L. crispatus</i> (A-2)	M		M	
<i>L. gallinarum</i> (A-4)			M	
<i>L. gasseri</i> (B-1)	M			+
<i>L. johnsonii</i> (B-2)	+	+	M	
<i>L. murinus</i>		?	?	M
<i>L. intestinalis</i>				
<i>L. salivarius</i>	M	M	M	
<i>L. agilis</i>		+	+	
<i>L. ruminis</i>	+			M
<i>L. vitulinus</i>	+			
<i>L. hamsteri</i>				
<i>L. aviarius</i>			+	
<i>L. casei</i>	+			
<i>L. reuteri</i>	M	M	M	M
<i>L. brevis</i>	+			

**Symbols: M Major component of *Lactobacilli* in Human Gastrointestinal Flora**  
**Questionable**

**Mitsuoka, T (1992) "The Human Gastrointestinal Flora"**  
**In *Lactic Acid Bacteria in Health and Disease*, Ed I, p. 76. Elsevier Applied Science.**

## NU study: Dirt's good for kids

Playing in, and even eating, dirt helps develop immune system, report says



Thom McDade sorts plasma samples at Northwestern University in Evanston. McDade participated in research that shows that kids who are exposed to dirt and germs have healthier hearts. (Andrew A. Nelles, Chicago Tribune / March 7, 2010)

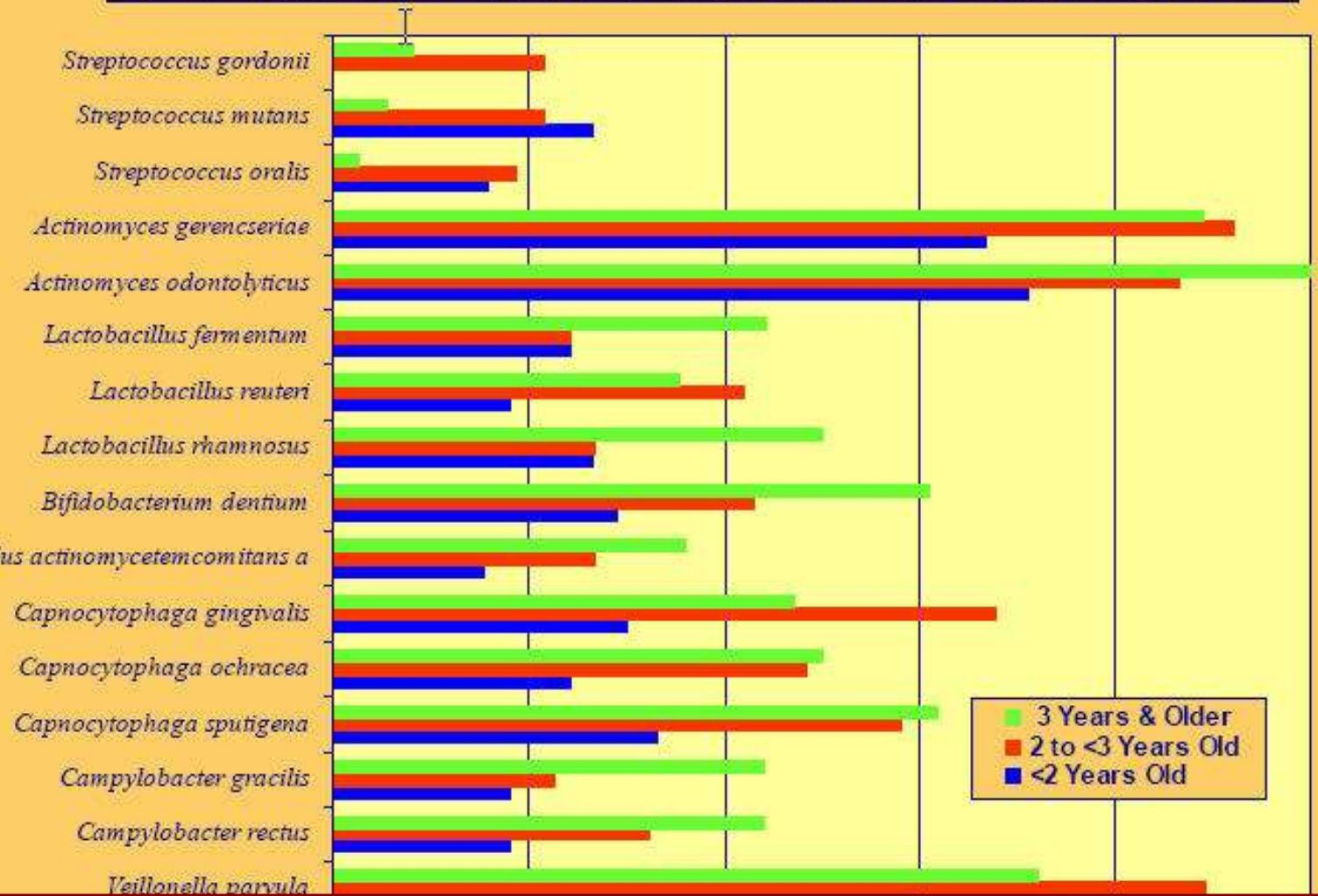


# *L. reuteri* is i

<2 Years Old	2-to<3 Year
--------------	-------------

<i>S. mutans</i>	<i>S. gordonii</i>
<i>S. oralis</i>	<i>S. mutans</i>
	<i>S. oralis</i>
	<i>L. buchnerii</i>
	<i>L. reuteri</i>
	<i>A. gerencseriae</i>
	<i>C. gingivalis</i>
	<i>C. ochracea</i>
	<i>C. sputigena</i>
	<i>V. parvula</i>
	<i>F. nuc. ss. nuc.</i>
	<i>F. nuc.</i>

**Figure 1: Age Differences in Microflora Among Caries-Free Children**



## Oral flora of caries-free 1-to-4-year-old children

M.E. Nunn<sup>1</sup>, A.C.R. Tanner<sup>2</sup>, S.C. Lu<sup>2</sup>, E. Kanasi<sup>2</sup>, N.R. Kressin<sup>3</sup>, H.K. Singh<sup>1</sup>, R.I. Garcia<sup>1</sup>

<sup>1</sup>Boston University School of Dental Medicine, Boston, MA

<sup>2</sup>The Forsyth Institute, Boston, MA <sup>3</sup>VA Medical Center, Bedford, MA

Reuter G. (200)



## ***L. reuteri* effect on infections in infants attending child care**

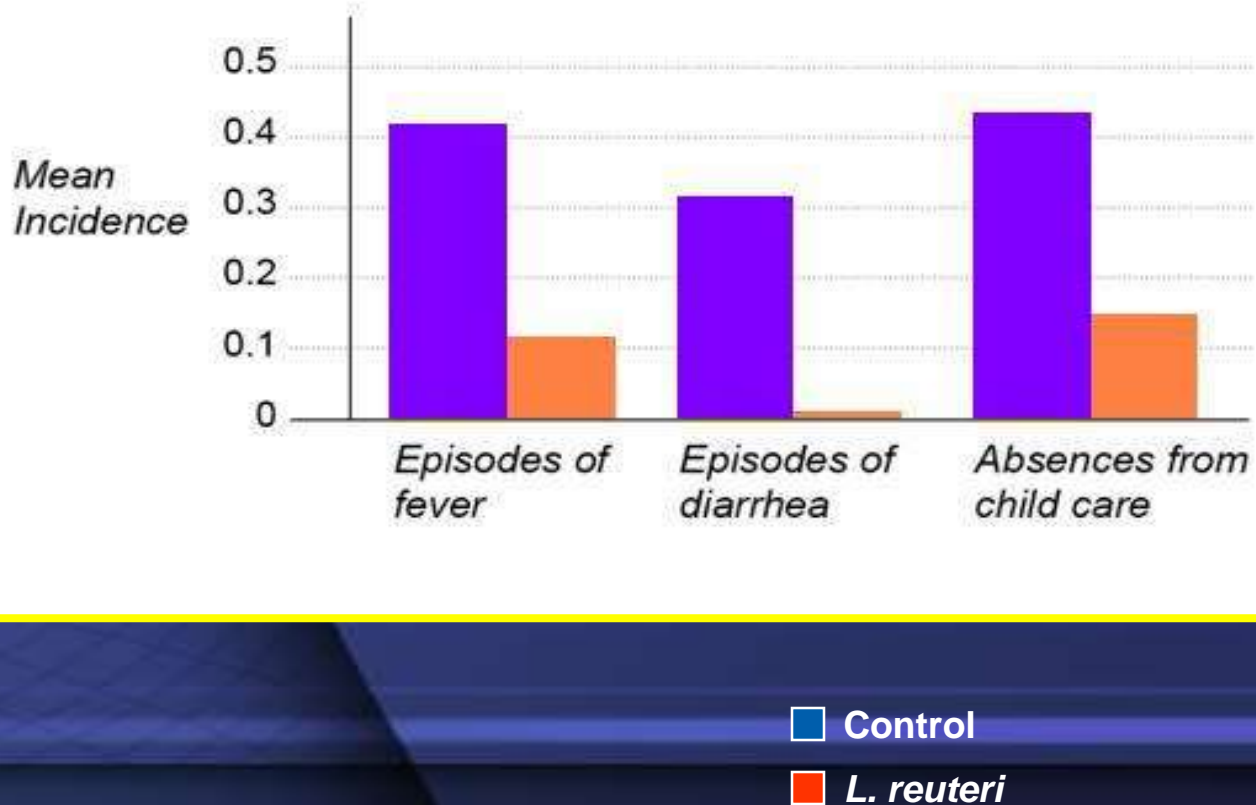
- *Results of a study by Weizman, Z. et al. (2005), Pediatrics: Effect of a probiotic infant formula on infections in child care centers: comparison of two probiotic agents.*
  - Study group: 201 healthy, full-term infants aged four to ten months were studied at 14 child care centers for 21 months, covering two winter and two summer seasons.





# *L. reuteri* effect on infections in infants attending child care

Effectiveness of *L. reuteri* Protectis on infections in child care centers





# ***L. reuteri* inhibits oral pathogenic bacteria**

- ***Aggregatibacter actinomycetemcomitans***
- ***Fusobacterium nucleatum***
- ***Porphyromonas gingivalis***
- ***Prevotella intermedia***
- ***Streptococcus mutans***



*L. reuteri* ATCC 55730  
*P. gingivalis* ATCC 33277  $10^7$  CFU/ml



*L. reuteri* PTA 5289  
*P. gingivalis* ATCC 33277  $10^7$  CFU/ml

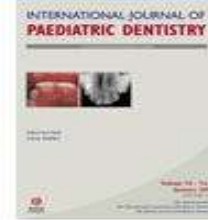
***L. reuteri* strains inhibits growth of *P. gingivalis***

Hedberg (2006), Nikawa (2004), Caglar (2006, 2007)



**To cite this article:** ESBER ÇAGLAR, OZGUR ONDER KUSCU, SULE KAVALOGLU CILDIR, SENEM SELUI KUVVETLI, NUKET SANDALLI (2008) A probiotic lozenge administered medical device and its effect on salivary mutans streptococci and lactobacilli

International Journal of Paediatric Dentistry 18 (1) , 35–39 doi:10.1111/j.1365-263X.2007.00866.x



## Abstract

### A probiotic lozenge administered medical device and its effect on salivary mutans streptococci and lactobacilli

ESBER ÇAGLAR, OZGUR ONDER KUSCU, SULE KAVALOGLU CILDIR, SENEM SELUI KUVVETLI & NUKET SANDALLI

Department of Paediatric Dentistry, Dental School, Yeditepe University, Istanbul, Turkey

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International Journal of Paediatric Dentistry 2008; 18: 35–39

## Abstract

**Background.** Previous studies have suggested that lactobacilli-derived probiotics in dairy products may affect oral ecology, but the effects of different delivery methods have received little attention.

**Aim.** The aim of the present study was to investigate the effect of the probiotic *Lactobacillus reuteri*, delivered by a new medical device, on the levels of salivary mutans streptococci and lactobacilli in young women with high *Streptococcus mutans* counts.

**Design.** This is a randomized, double-blind, placebo-controlled study involving 20 healthy young women (aged 20 years): 10 as subjects and 10 as controls. The study subjects (Group A) sucked the medical device containing the probiotic lozenge with *L. reuteri* ATCC 55730/*L. reuteri* ATCC PTA 5289 ( $1.1 \times 10^8$  CFU) once daily for 10 days, while the control subjects (Group B) received placebo medical devices without bacteria. Salivary mutans streptococci and lactobacilli were enumerated with chair-side kits at baseline and 1 day after the final ingestion.

**Results.** Salivary *S. mutans* levels in the probiotic test group were significantly reduced, with statistical significance of reduction ( $P < 0.05$ ).

**Conclusions.** A short-term daily ingestion of lactobacilli-derived probiotics delivered via medical device containing probiotic lozenge reduced the levels of salivary mutans.

plasmids from  
and  
daughter strain *L.*

y, and Stefan Roos\*  
h University of  
5, SE-750 07  
Box 3242, SE - 103

S.



# Foods with natural presence of *L. reuteri*



All Lifeway Kefir products contain 25 – 30 billion Colony Forming Units (CFU) of live and active kefir cultures per cup. The kefir cultures include:

Lactobacillus Lactis  
Lactobacillus Rhamnosus  
Streptococcus Diacetylactis  
Lactobacillus Plantarum  
Lactobacillus Casei  
Saccharomyces Florentinus  
Leuconostoc Cremoris  
Bifidobacterium Longum  
Bifidobacterium Breve  
Lactobacillus Acidophilus  
Bifidobacterium Lactis\*  
Lactobacillus Reuteri\*

\*Our Frozen Kefir products do not contain Bifidobacterium Lactis or Lactobacillus Reuteri





# Probiotic Research

Journal of  
Indian Society of  
Periodontology

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Current issue  
Instructions  
Submit article

J Indian Soc Periodontol. 2011 Jan-Mar; 15(1): 23–28.

doi: [10.4103/0972-124X.82260](https://doi.org/10.4103/0972-124X.82260)

## Probiotics in periodontal health and disease

Table 2

### Periodontal clinical studies done on probiotics

Strain	Mode	Result	Author/Year
<i>L. acidophilus</i>	Tablet "acilant"	Replacement of gram negative flora with gram positive and lactobacillus	Pozharitskaia <i>et al.</i> <sup>[17]</sup> 1994
Mixture of probiotics	Tablet	Improved clinical signs of periodontal disease	Grudianov <i>et al.</i> 2002
<i>L. helveticus</i>	<i>In vitro</i>	Reduction in clinical finding of gingivitis and periodontitis	Narva <i>et al.</i> 2004
<i>L. casei</i>	Periodontal dressing	Release short peptide that stimulates bone formation	Volozhin <i>et al.</i> 2004
<i>L. reuteri</i>	Specific study formulation	Decrease remission period	Krasse <i>et al.</i> <sup>[11]</sup> 2005
<i>W. cibaria</i>	Gargle	Decrease gingivitis and periodontitis scores	Kang <i>et al.</i> 2006
		Decrease VSC production and co-aggregation of <i>F. nucleatum</i>	
<i>S. sanguis</i> , <i>S. salavaris</i> , <i>S. mitis</i>	Application after root planning	Reduce sub gingival periopathogens	Teughelus <i>et al.</i> 2007
<i>S. sanguis</i> , <i>S. salavaris</i> , <i>S. mitis</i>	<i>In vitro</i>	Inhibition of aggregatibacter actinobacillucomitans	Teughelus <i>et al.</i> 2007
<i>L. salivaris</i>	Tablet	Clinical sign improvement in smoker and non-smokers	Shimauchi <i>et al.</i> 2008
<i>L. brevis</i>	Lozenges	Decrease PGE2 and MMP	Ricca <i>et al.</i> 2001
<i>L. reutri</i>	Chewing gum	Decrease proinflammatory cytokines in GCF	Twetman <i>et al.</i> <sup>[8]</sup> 2009

“There is no published study showing evidence supporting probiotics”



# Probiotic Research



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*PLoS One*. 2017; 12(10): e0187258.

Published online 2017 Oct 30. doi: [10.1371/journal.pone.0187258](https://doi.org/10.1371/journal.pone.0187258)

PMCID: PMC5662169

PMID: [29084251](https://pubmed.ncbi.nlm.nih.gov/29084251/)

## Is yogurt intake associated with periodontitis due to calcium?

[Hye-Sung Kim](#), Conceptualization, Writing – original draft, [Young-Youn Kim](#), Conceptualization, Formal analysis, Methodology, [Jeong-Kyu Oh](#), Conceptualization, Formal analysis, Methodology, and [Kwang-Hak Bae](#), Conceptualization,

In conclusion, periodontitis was significantly associated with less intake of yogurt among the Korean adults, but the calcium contained in yogurt is not likely to cause it.



# Teen and Young Adult High risk pregnancies

- Teen age pregnancy and Periodontal Disease



*Infect Immun.* 2009 Jul;77(7):3075-9. doi: 10.1128/IAI.00209-09. Epub 2009 Apr 27.

## Complementation of the *fadA* mutation in *Fusobacterium nucleatum* demonstrates that the surface-exposed adhesin promotes cellular invasion and placental colonization.

Ikegami A<sup>1</sup>, Chung P, Han YW.

### ⊕ Author information

Promotes cell invasion and colonization

### Abstract

*Fusobacterium nucleatum* is a gram-negative oral anaerobe implicated in periodontal disease and adverse pregnancy outcome. The organism colonizes the mouse placenta, causing localized infection and inflammation. The mechanism of placental colonization has not been elucidated. Previous studies identified a novel adhesin from *F. nucleatum*, FadA, as being involved in the attachment and invasion of host cells. The *fadA* deletion mutant *F. nucleatum* 12230 US1 was defective in host cell attachment and invasion in vitro, but it also exhibited pleiotropic effects with altered cell morphology and growth rate. In this study, a *fadA*-complementing clone, *F. nucleatum* 12230 USF81, was constructed. The expression of FadA on USF81 was confirmed by Western blotting and immunofluorescent labeling. USF81 restored host cell attachment and invasion activities. The ability of *F. nucleatum* 12230, US1, and USF81 to colonize the mouse placenta was examined. US1 was severely defective in placental colonization compared to the wild type and USF81. Thus, FadA plays an important role in *F. nucleatum* colonization in vivo. These results also represent the first complementation studies for *F. nucleatum*. FadA may be a therapeutic target for preventing *F. nucleatum* colonization of the host.

PMCID: PMC375172

## Ilbirths in Pregnant

[nas S. McCormick<sup>4</sup>](#)



# Colorectal Cancer- *Fusibacterium nucleatum*



## International Cancer Screening Network

Sponsored by the National Cancer Institute

### North America

Canada	45.4	14.4	31.8	9.4
United States	34.1	9.9	25.0	7.7

## ***Fusobacterium nucleatum* Promotes Colorectal Carcinogenesis by Modulating E-Cadherin/ $\beta$ -Catenin Signaling via its FadA Adhesin**

Mara Roxana Rubinstein<sup>7</sup>, Xiaowei Wang<sup>7</sup>, Wendy Liu, Yujun Hao, Guifang Cai, Yiping W. Han 

### Summary

*Fusobacterium nucleatum* (Fn) has been associated with colorectal cancer (CRC), but causality and underlying mechanisms remain to be established. We demonstrate that Fn adheres to, invades, and induces oncogenic and inflammatory responses to stimulate growth of CRC cells through its FadA adhesin. FadA binds to E-cadherin, activates  $\beta$ -catenin signaling, and differentially regulates the inflammatory and oncogenic responses. The FadA-binding site on E-cadherin is mapped to an 11-amino-acid region. A synthetic peptide derived from this region of E-cadherin abolishes FadA-induced CRC cell growth and oncogenic and inflammatory responses. The *fadA* gene levels in the colon tissue from patients with adenomas and adenocarcinomas are >10–100 times higher compared to normal individuals. The increased FadA expression in CRC correlates with increased expression of oncogenic and inflammatory genes. This study unveils a mechanism by which Fn can drive CRC and identifies FadA as a potential diagnostic and therapeutic target for CRC.



# Pre-natal intervention

*ClinicalTrials.gov*

## **Saving Lives at Birth: Primary Prevention of Periodontal Disease in Relation to Preterm Birth in**

**This study is currently recruiting participants. (see [Contacts and Locations](#))**

*Verified July 2016 by Kjersti Aagaard, Baylor College of Medicine*

**Sponsor:**  
Baylor College of Medicine

ClinicalTrials.gov Identifier:  
NCT02333227

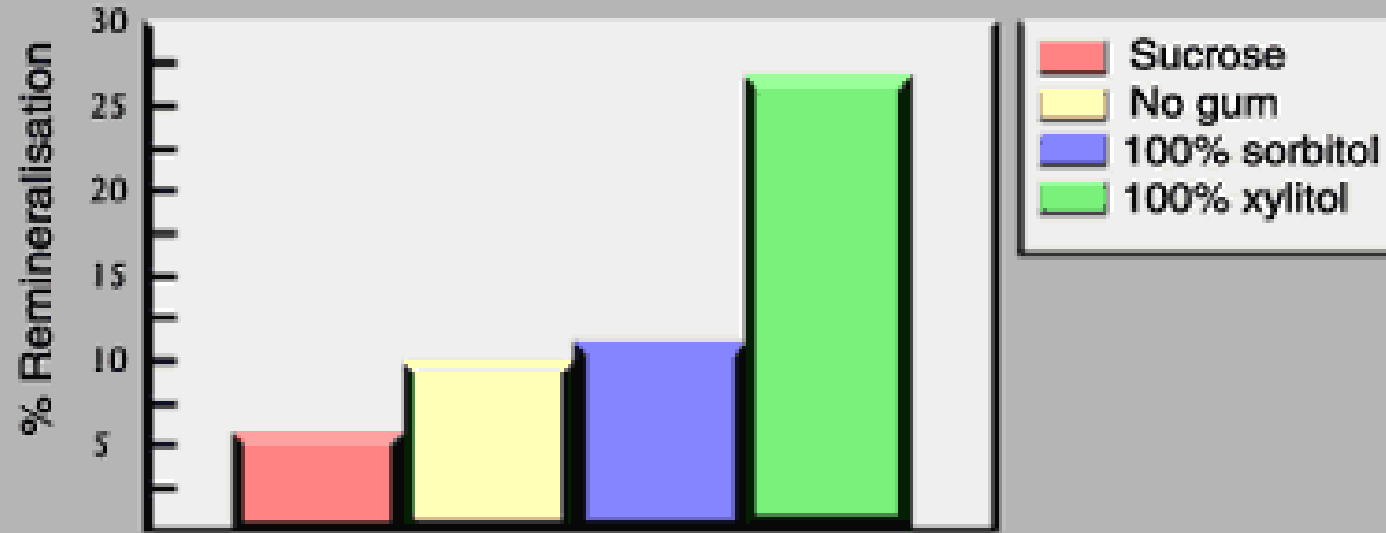
First received: December 23, 2014  
Last updated: July 18, 2016  
Last verified: July 2016



In rodents, subcutaneous inoculations with periodontal pathogens cause dose-dependent decreases in pup weights, and elicit inflammatory responses that can trigger preterm birth when present in amniotic fluid. Periodontitis (defined as a destructive inflammatory disease of the periodontium) has a prevalence of **30% or greater in women** of child bearing age. By this definition, it involves microbial infiltration of the periodontium, which stimulates a host inflammatory response, recurrent bacteremia, and the production of cytokines and prostaglandins which trigger risk of preterm birth. It is the same production of prostaglandins which are felt to mediate the risk of preterm birth. **The investigators' overarching hypothesis is that comprehensive primary preterm birth prevention, inclusive of maternal oral health with xylitol chewing gum (the intervention), will reduce the rate of periodontal disease and caries, preterm birth prevalence, and neonatal mortality.**



# Xylitol- research



dy.

PL.

Arbor 48109-1078 USA.

## Belize Study

and consumes considerable health care resources. The chewing of No clinical study has simultaneously investigated the effectiveness with double-blind cohort in Central America. One thousand subjects (no supervised gum consumption of 100% xylitol gum was supervised. Four countries have reported the development of a marginal increase in

confidence interval, 0.96 to 1.49;  $p = 0.1128$ ). Sorbitol gum significantly reduced caries rates (relative risk = 0.0074). The four xylitol gums were most effective in reducing caries rates, the most effective agent (relative risk = 0.27; 95% confidence interval, 0.20 to 0.36;  $p = 0.0001$ ). This gum was superior to any other gum ( $p < 0.0001$ ). This gum was not significantly more effective than xylitol, but they reduced caries rates significantly compared with the no-gum group. DMX conclusions. The results suggest that systematic usage of polyol-based chewing gums reduces caries being more effective than sorbitol gums.





# Xylitol- research

J Dent Res. 2000 Mar;79(3):882-7.

## Influence of maternal xylitol consumption on acquisition of mutans streptococci by infants.

Söderling E, Isokangas P, Pienihäkkinen K, Tenovou J.

Institute of Dentistry, University of Turku, Finland. eva.soderling@utu.fi

classic

2000

### Abstract

Xylitol is effective as a non-cariogenic sugar substitute. Habitual xylitol consumption appears to select for mutans streptococci (MS) with impaired adhesion properties, i.e., they shed easily to saliva from plaque. One hundred sixty-nine mother-child pairs participated in a two-year study exploring whether the mothers' xylitol consumption could be used to prevent mother-child transmission of mutans streptococci. All mothers showed high salivary levels of mutans streptococci during pregnancy. The mothers in the xylitol group (n = 106) were requested to chew xylitol-sweetened gum (65% w/w) at least 2 or 3 times a day, starting three months after delivery. In the two control groups, the mothers received either chlorhexidine (n = 30) or fluoride (n = 33) varnish treatments at 6, 12, and 18 months after delivery. The children did not chew gum or receive varnish treatments. MS were assessed from the mothers' saliva at half-year intervals and from the children's plaque at the one- and two-year examinations. The MS were cultured on Mitis salivarius agars containing bacitracin. The salivary MS levels of the mothers remained high and not significantly different among the three study groups throughout the study. At two years of age, 9.7% of the children in the xylitol, 28.6% in the chlorhexidine, and 48.5% in the fluoride varnish group showed a detectable level of MS. In conclusion, therefore, habitual xylitol consumption by mothers was associated with a statistically significant reduction of the probability of mother-child transmission of MS assessed at two years of age. The effect was superior to that obtained with either chlorhexidine or fluoride varnish treatments performed as single applications at six-month intervals.

Mutans strep detectable-  
9.7% of xylitol group  
28.6% of chlorhexidine group  
48.5% of fluoride varnish group





# Xylitol- research

Eur J Dent. 2011 Jan;5(1):24-31.

## The effect of xylitol on the composition of the oral flora: a pilot study.

Söderling E, Hirvonen A, Karjalainen S, Fontana M, Catt D, Seppä L.

Adjunct Professor, Institute of Dentistry, University of Turku, Finland. eva.soderling@utu.fi

2011

### Abstract

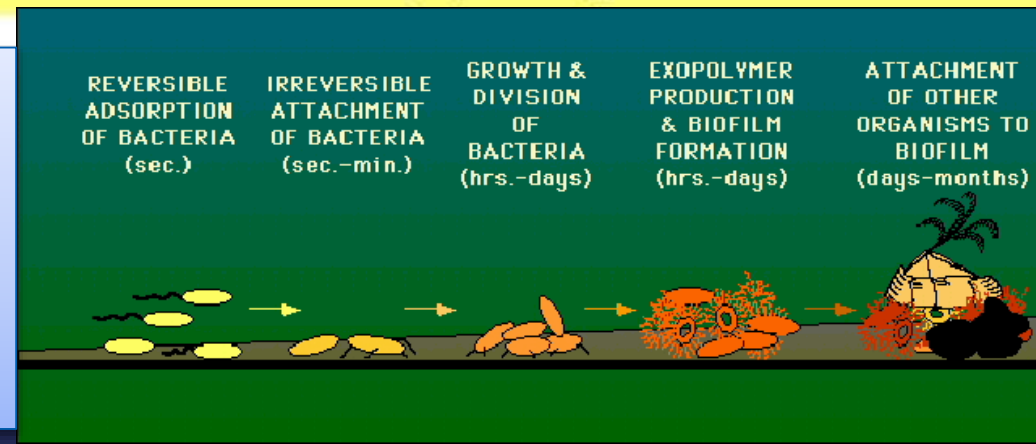
**OBJECTIVES:** Our aim was to investigate the effect of short-term xylitol consumption on the microbial composition of plaque and saliva.

**METHODS:** Twelve volunteers (22-38 yrs) harboring mutans streptococci (MS) participated in the randomized, double-blind, cross-over study. The experimental chewing gum contained 65% xylitol while the control gum contained 63% sorbitol and 2% maltitol w/w. The polyol dose was approximately 6 g/day. Stimulated saliva and plaque samples were collected before and after the two four-week test periods. The samples were cultured for MS, total streptococci, lactobacilli, and total facultatives. A part of the samples were subjected to DNA-DNA hybridizations of 14 microbial plaque species: *Actinomyces naeslundii*, *A. viscosus*, *Fusobacterium nucleatum*, *Lactobacillus acidophilus*, *L. fermentum*, *L. paracasei*, *L. rhamnose*, *L. plantarum*, *Streptococcus gordonii*, *S. oralis*, *S. parasanguis*, *S. salivarius*, *S. sanguinis*, *Veillonella parvula*.

**RESULTS:** The MS counts of the plaque samples collected from "caries-prone" tooth sites decreased significantly ( $P < .01$ ) in the xylitol gum group but not in the sorbitol gum group. Also the plaque MS percentage decreased significantly in the xylitol gum group ( $P < .01$ ). The salivary MS counts did not decrease either in the xylitol or in the sorbitol gum groups. Nor were changes detected in the salivary levels of total streptococci or lactobacilli. The DNA-DNA hybridization assay revealed no study-induced changes in the microbial composition of the dental plaque.

**CONCLUSIONS:** Within the limitations of this pilot study, xylitol consumption reduced MS counts in plaque but appeared not to affect the microbial composition of plaque or saliva in general.

Smart targeting- negligible effect on many probiotic bacteria while greatly reducing mutans streptococci.





ucts

- Xlear (Clear)

# WHISKEY Tooth Paste!



Genuine 6 Proof Stuff  
**SCOTCH • BOURBON**

Why fight oral hygiene—enjoy it!  
Here's real he-man toothpaste, best  
argument yet for brushing 3 times a  
day. 2½ oz. tubes flavored with the  
real thing—Scotch or Bourbon.  
Night-before feeling on the morning  
after. Rinse with soda in-  
stead of water if you prefer.  
Per tube ppd. . . . .

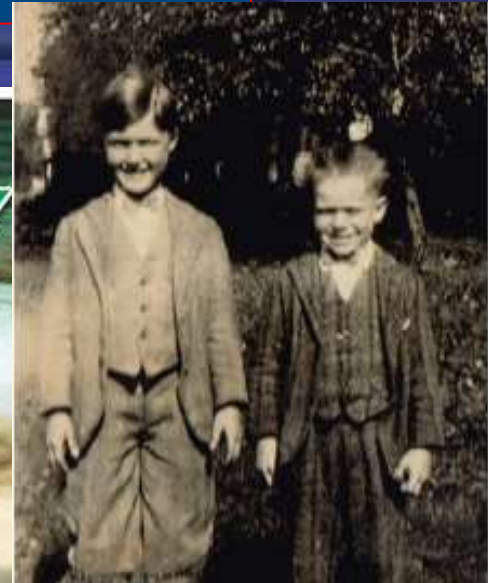
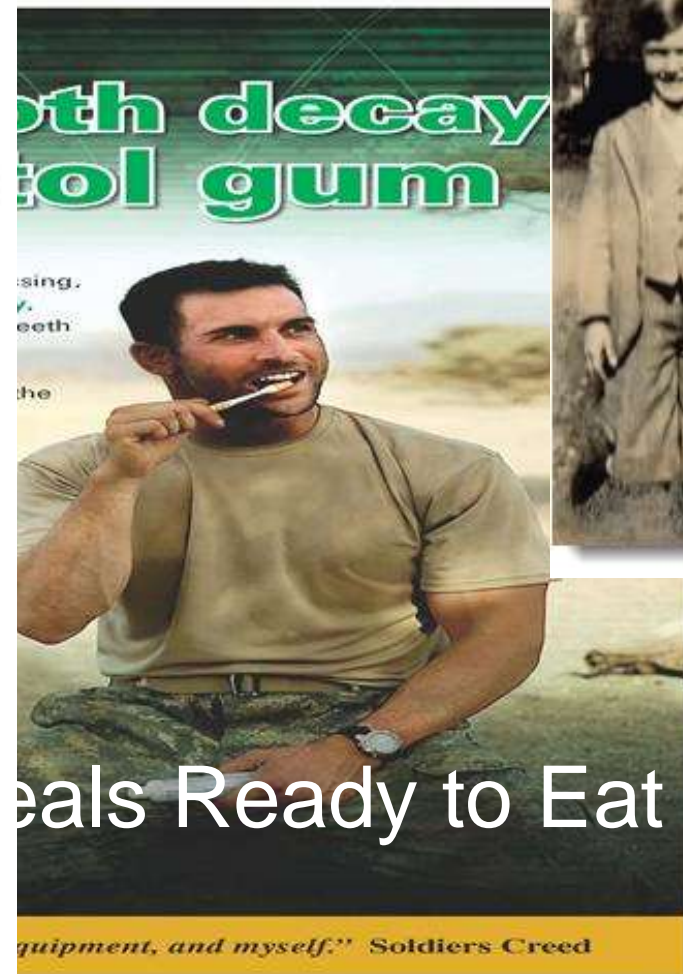
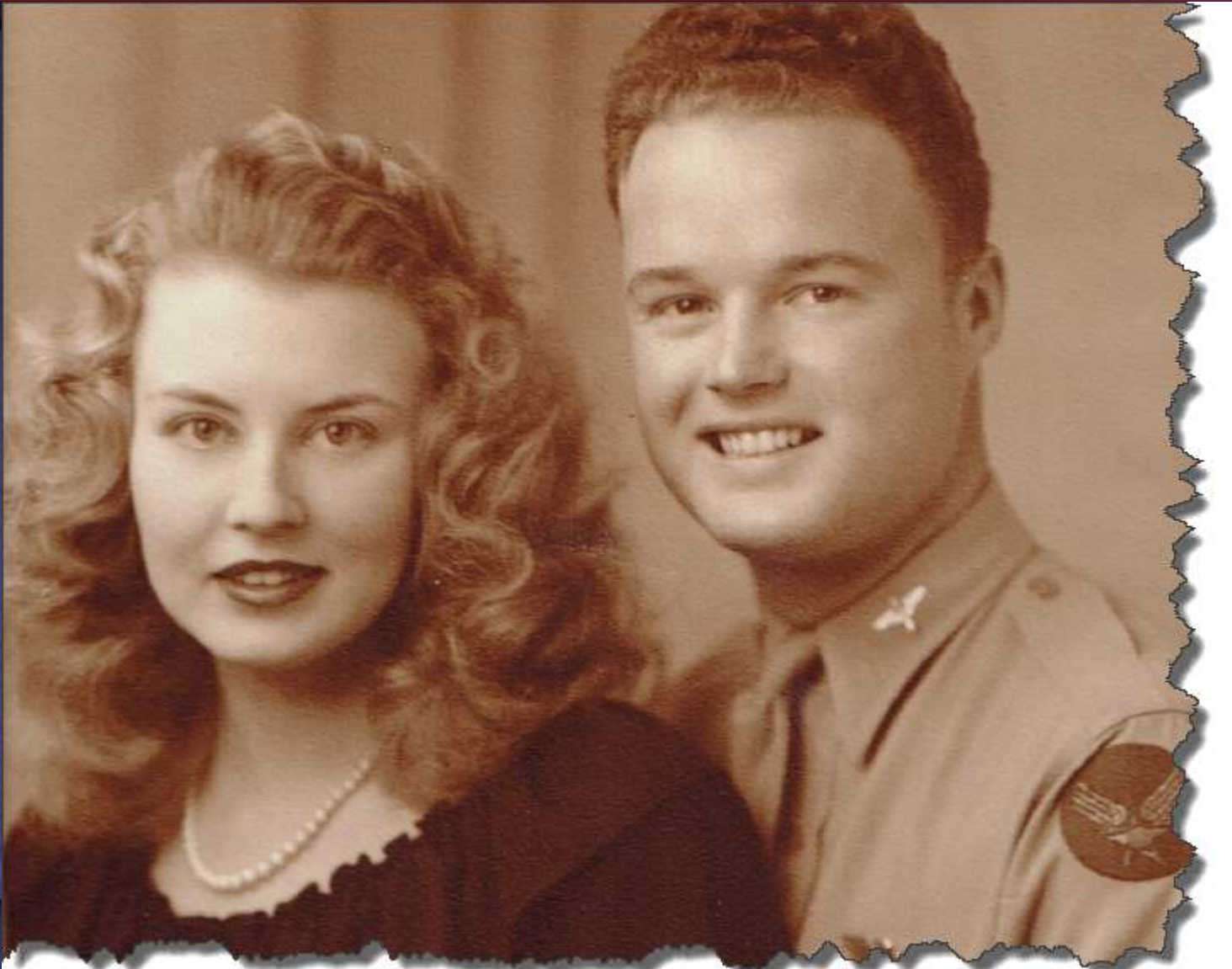
**\$1**

**Greenland Studios**  
DEPT. HG-114, MIAMI 47, FLORIDA





# MRE- xylitol gum- G.I. issue



Not the tastiest food



# New Xylitol Products



## For Special Needs!

- Children's Mouthwash- safe for all ages



# Xylitol and Dentistry

PEDIATRIC DENTISTRY V 39 I NO 2 MAR / APR 17



SYSTEMATIC REVIEW AND META-ANALYSIS



## Effectiveness of Xylitol in Reducing Dental Caries in Children

Abdullah A. Marghalani, BDS, MSD, DrPH<sup>1</sup> • Emilie Guinto, DDS<sup>2</sup> • Minhthu Phan, DDS<sup>3</sup> • Vineet Dhar, BDS, MDS, PhD<sup>4</sup> • Norman Tinanoff, DDS, MS<sup>5</sup>

- Scheinin A, Mäkinen KK, Tammisalo E, Rekola M. **Turku sugar studies**. XVIII. Incidence of dental caries in relation to 1-year consumption of xylitol chewing gum. Acta Odontol Scand 1975;33(5):269-78.
  - Scheinin A, Mäkinen KK, Ylitalo K. **Turku sugar studies**. V. Final report on the effect of sucrose, fructose and xylitol diets on caries incidence in man. Acta Odontol Scand 1976;34(4):179-216.
- DMI 5/1, respectively).





# Xylitol and Dentistry

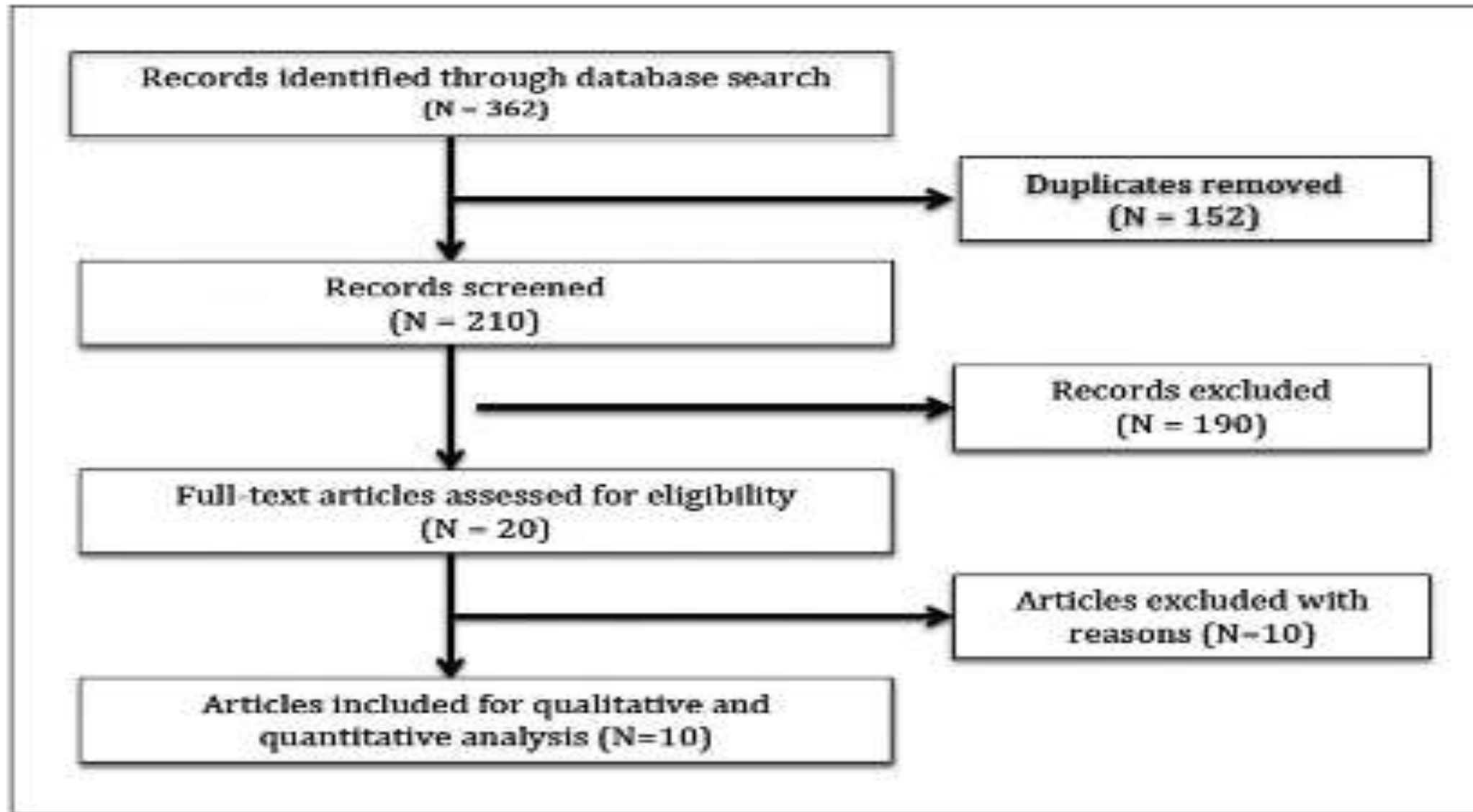


Figure 1. Flowchart of the trial selection process and elimination of studies.

2 MAR 17 APR 17



DDS, MS

All  
and  
r?







## Effectiveness of Xylitol in Reducing Dental Caries in Children

Abdullah A. Marghalani, BDS, MSD, DrPH<sup>1</sup> • Emilie Guinto, DDS<sup>2</sup> • Minhthu Phan, DDS<sup>3</sup> • Vineet Dhar, BDS, MDS, PhD<sup>4</sup> • Norman Tinanoff, DDS, MS<sup>5</sup>

- Toothpaste works, wipes work, but gum had a “varied” result.

Xylitol dentifrice-840/840 Control dentifrice-837/837	3 years from when the children were 7-12 years old	Significant reduction in DFS 5.0±3.7 vs. 5.7±4.1	High risk
Xylitol dentifrice-1280/1280 Control dentifrice-1259/1259	30 months from when the children were 7-12 years old	Significant reduction in DFS 1.30±1.89 vs. 1.51±2.00 and in DFT 0.69±1.10 vs. 0.81±1.21	High risk





- Stecken-Blicks (sic) changes the Forest plot of results and Alanen- inconsistent!

**The fluoride content in the pipe drinking water was  $\leq 0.3$  p.p.m. !!!**

One hundred and sixty healthy 10- to 12-year-old children with high caries risk were selected. After informed consent, they were randomly assigned into a **xylitol** and a **xylitol/ fluoride group**. They were instructed to take two tablets three times a day (total xylitol and fluoride dose 2.5 g and 1.5 mg, respectively). The dropout rate was 28%, and 41% exhibited a good **compliance** with the study protocol. No statistically significant differences in caries incidence could be found between the study groups ( $P > 0.05$ ). **Huge non-compliance rate of 59%! Plus 28% dropout??**





• T

ath




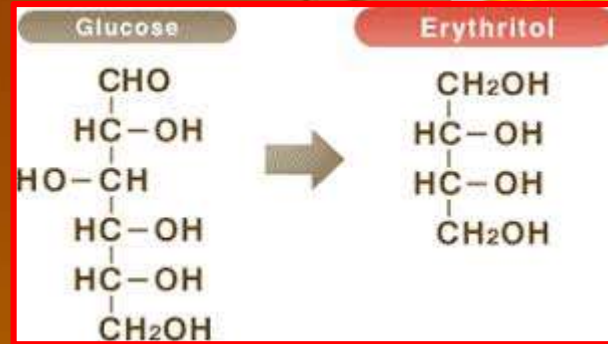


# Probiotics and Microbiome

## NUTRITION™

Erythritol is a sweet antioxidant

Gertjan J.M. den Hartog, Ph.D., , Agnes W. Boots, Ph.D., Aline Adam-Perrot, Ph.D.<sup>†</sup>, Fred Brouns, Ph.D., Inge W.C.M. Verkooijen, M.Sc., Antje R. Weseler, Ph.D., Guido R.M.M. Haenen, Ph.D., Aalt Bast,



Erythritol was shown to be an excellent HO• radical scavenger and an inhibitor of 2,2'-azobis-2-amidinopropane dihydrochloride–induced hemolysis but inert toward superoxide radicals. The reaction of erythritol with hydroxyl radicals resulted in the formation of erythrose and erythrulose by abstraction of a carbon-bound hydrogen atom. Erythritol displayed an endothelium-protective effect and, in accordance with the in vitro experiments, erythrose was found in the urine of erythritol-consuming rats.





# Xylitol- “Prebiotic”



## Prebiotic potential of L-sorbose and xylitol in promoting the growth and metabolic activity of specific butyrate-producing bacteria in human fecal culture FREE

Tadashi Sato ✉, Shiro Kusuvara, Wakae Yokoi, Masahiko Ito, Kouji Miyazaki

*FEMS Microbiology Ecology*, Volume 93, Issue 1, 1 January 2017, fiw227, <https://doi.org>

- Dietary low-digestible carbohydrates (LDCs) affect gut microbial metabolism, including the production of short-chain fatty acids. Fecal suspensions from five healthy males were anaerobically incubated with various LDCs. L-Sorbose and xylitol markedly promoted butyrate formation in cultures. Bacterial 16S rRNA gene-based denaturing gradient gel electrophoresis analyses of these fecal cultures revealed a marked increase in the abundance of bacteria closely related to the species *Anaerostipes hadrus* or *A. caccae* or both, during enhanced butyrate formation from L-sorbose or xylitol.



# Polyols- “Prebiotics”

molecular oral  
microbiology

Original Article

Erythritol alters microstructure and metabolomic profiles of biofilm composed of *Streptococcus gordonii* and *Porphyromonas gingivalis*

E. Hashino, M. Kuboniwa ✉, S.A. Alghamdi, M. Yamaguchi, R. Yamamoto, H. Cho, A. Amano

Metabolome analyses using capillary electrophoresis time-of-flight mass spectrometry revealed that a number of nucleic intermediates and constituents of the extracellular matrix, such as nucleotide sugars, were decreased by erythritol in a dose-dependent manner.





# Polyols- “Prebiotics”

[J Periodontol. 2014 Jun; 85\(6\): e212–e223.](#)

PMID

Published online 2014 Mar 4. doi: [10.1902/jop.2014.130455](#)

Xylitol, an Anticaries Agent, Exhibits Potent Inhibition of Inflammatory Responses in Human THP-1-Derived Macrophages Infected With *Porphyromonas gingivalis*

[Eunjoo Park](#),\* [Hee Sam Na](#),\* [Sheon Min Kim](#),\* [Shannon Wallet](#),† [Seunghee Cha](#),‡ and [Jin Chung](#)\*

- The pretreatment of xylitol significantly inhibited the *P. gingivalis*– induced cytokines production and nitric oxide production. In addition, **xylitol inhibited the attachment of live *P. gingivalis* on THP-1-derived macrophages.** Furthermore, xylitol exerted anti-phagocytic activity against both *Escherichia coli* and *P. gingivalis*.



# Polyols- “Prebiotics”

JOURNAL OF  
Periodontology



Discovery Science

## *Aggregatibacter actinomycetemcomitans*-Induced AIM2 Inflammasome Activation Is Suppressed by Xylitol in Differentiated THP-1 Macrophages

Seyeon Kim, Mi Hee Park, Yu Ri Song, Hee Sam Na, Jin Chung✉

First published: 01 June 2016 | <https://doi.org/10.1902/jop.2016.150477> | Cited by: 5

- *A. actinomycetemcomitans* induced IL-1 $\beta$  production and AIM2 inflammasome activation. **Xylitol inhibited these effects**, possibly by suppressing internalization of *A. actinomycetemcomitans* into cells. Thus, this study proposes a mechanism for IL-1 $\beta$  production via inflammasome activation and discusses a **possible use for xylitol in periodontal inflammation caused by *A. actinomycetemcomitans***.



# Take Home- probiotics



- Polyols can be considered as “prebiotics”
- Polyols are anti-inflammatory also
- Polyols inhibit pathogens



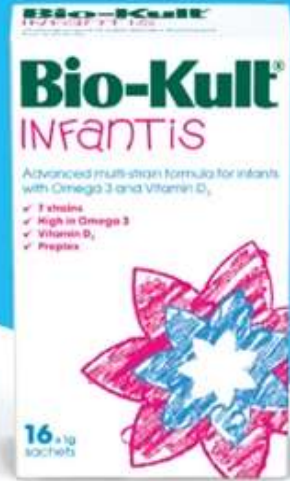
# Prebiotics and Probiotics

- Oral Products for Oral Health





# Infant Probiotics- Necessary?



## FROM BIRTH AND BEYOND

Look after your child's immune system\*  
with Bio-Kult Infantis!

\*Contains vitamin D<sub>3</sub> to contribute to the normal function of the immune system.



- Depends on the maternal microbiome- probably YES!



# Probiotics- BioGaia Protectis

- BioGaia Protectis contains the Lactobacillus 17938) that helps maintain natural balance. L. reuteri Protectis has strong adaptability. L. reuteri Protectis clinical trials for children.



element

L. reuteri DSM

restore a

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humans. L.

than 130

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# Probiotics and Microbiome

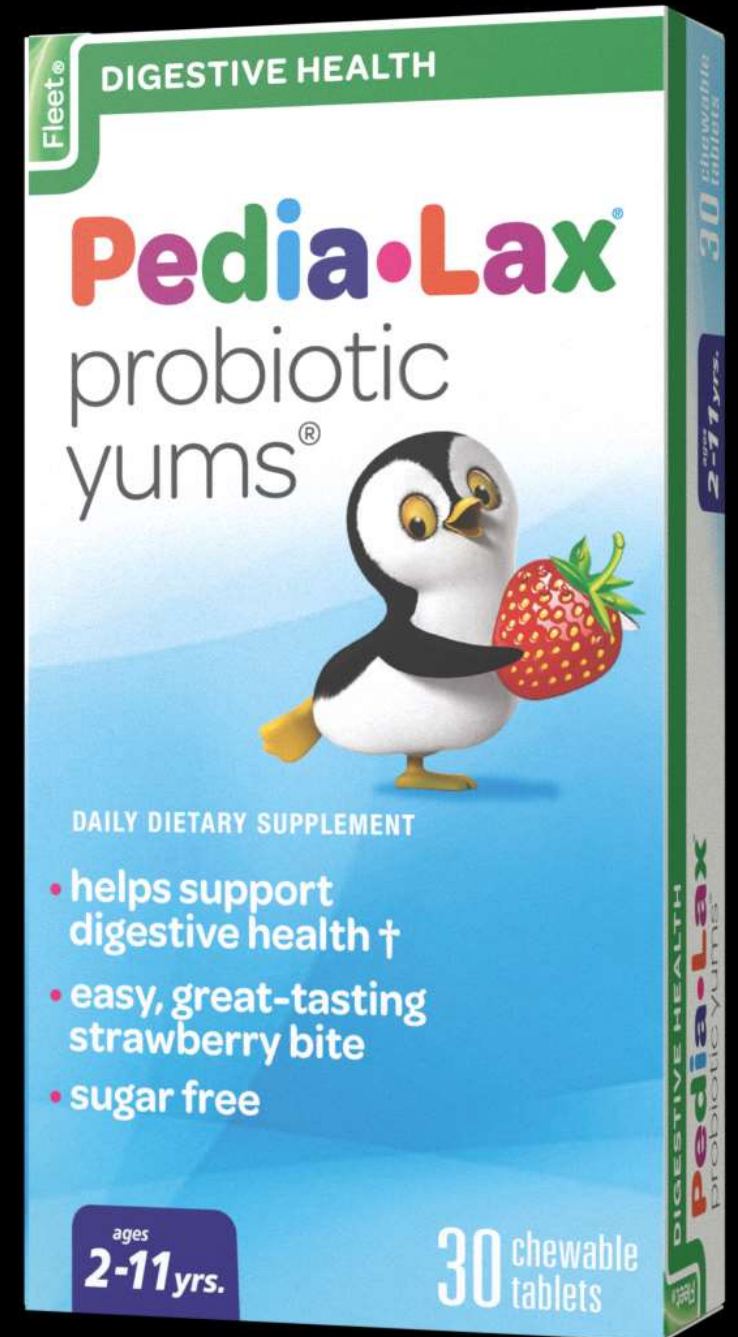
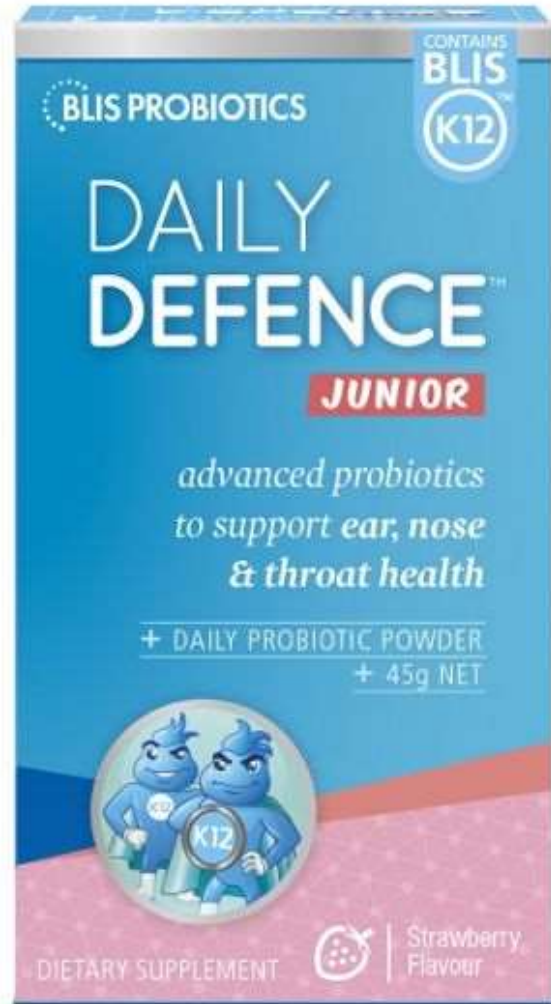
- Dental Caries- “an epidemic”
- Periodontal Pathogens and Systemic Disease  
- “tragic”





# Prebiotics and Probiotics

## Oral Products for Oral Health





# Prebiotics and Probiotics

- **The Microbiome**
- Responds to the climate, diet, exercise, and all other environmental influences. Not **STATIC!**

But may  
resist  
change!  
Just like  
you and me



# Take Home- probiotics



- Most probiotics are worthless, not tested, wrong strains and not sufficient in quantity.





# Cervitec Plus- Ivoclar

- FDA approved in 2008
- Used in Europe for many years
- **1% chlorhexidine and 1% thymol varnish**





# Cervitec Plus- Ivoclar

- Swollen and inflamed gingival tissues
- Periodontal Classification Type I- gingivitis





# Cervitec Plus- Ivoclar

Unit dose and  
bulk  
packaging





# Cervitec Plus- Ivoclar

## Inside Dentistry

June 2011, Volume 7, Issue 6

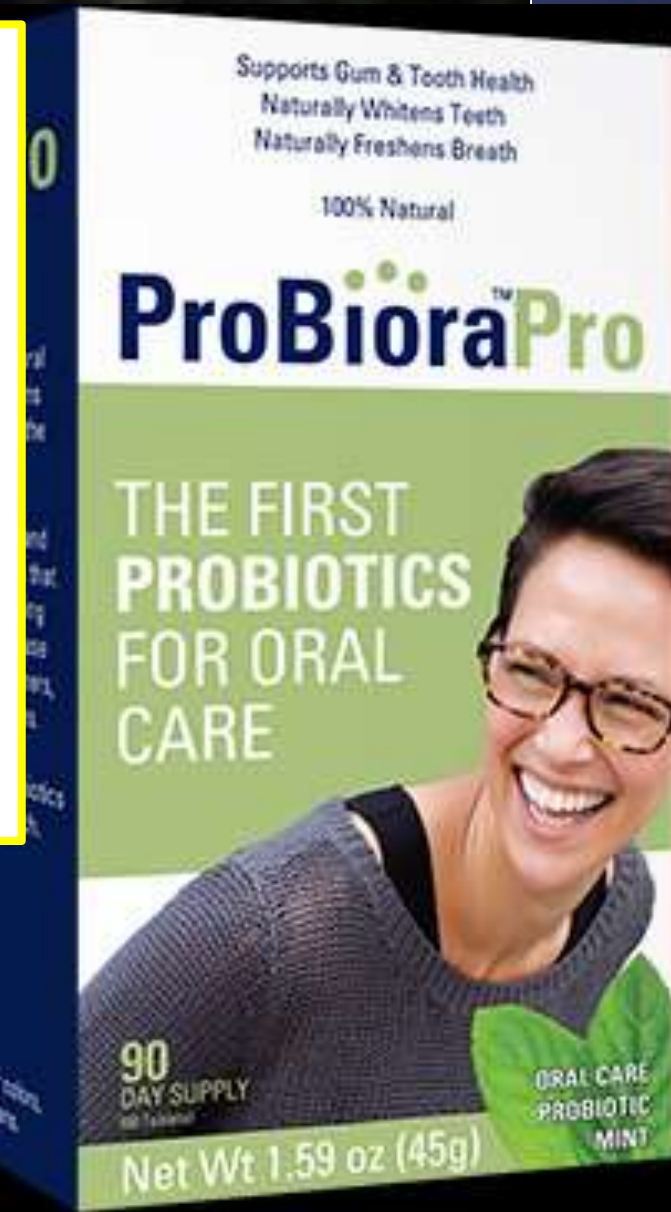
Published by AEGIS Communications

## Clinical Application of Probiotic Therapy

New adjunctive therapies offer new alternatives for treatment.

By Mark L. Cannon, DDS, MS

then start a prob





# **DNA-PCR and CRT Results in Children After Probiotic use**

**THE PRIMARY OBJECTIVE OF THIS  
CLINICAL STUDY IS TO DETERMINE  
THE EFFECT, IF ANY, OF “OVER THE  
COUNTER” PROBIOTIC  
SUPPLEMENTS ON THE DNA-PCR  
And CRT ANALYSIS**





# DNA-PCR and CRT Results in Children After Probiotic use

## Methods

- 60 patients 6 to 12 years of age- caries prone with 4 or more restorations and /or lesions
- CRT collected before and after probiotic use
- 8 week (60 day) experimental time period- considered optimal to see effect

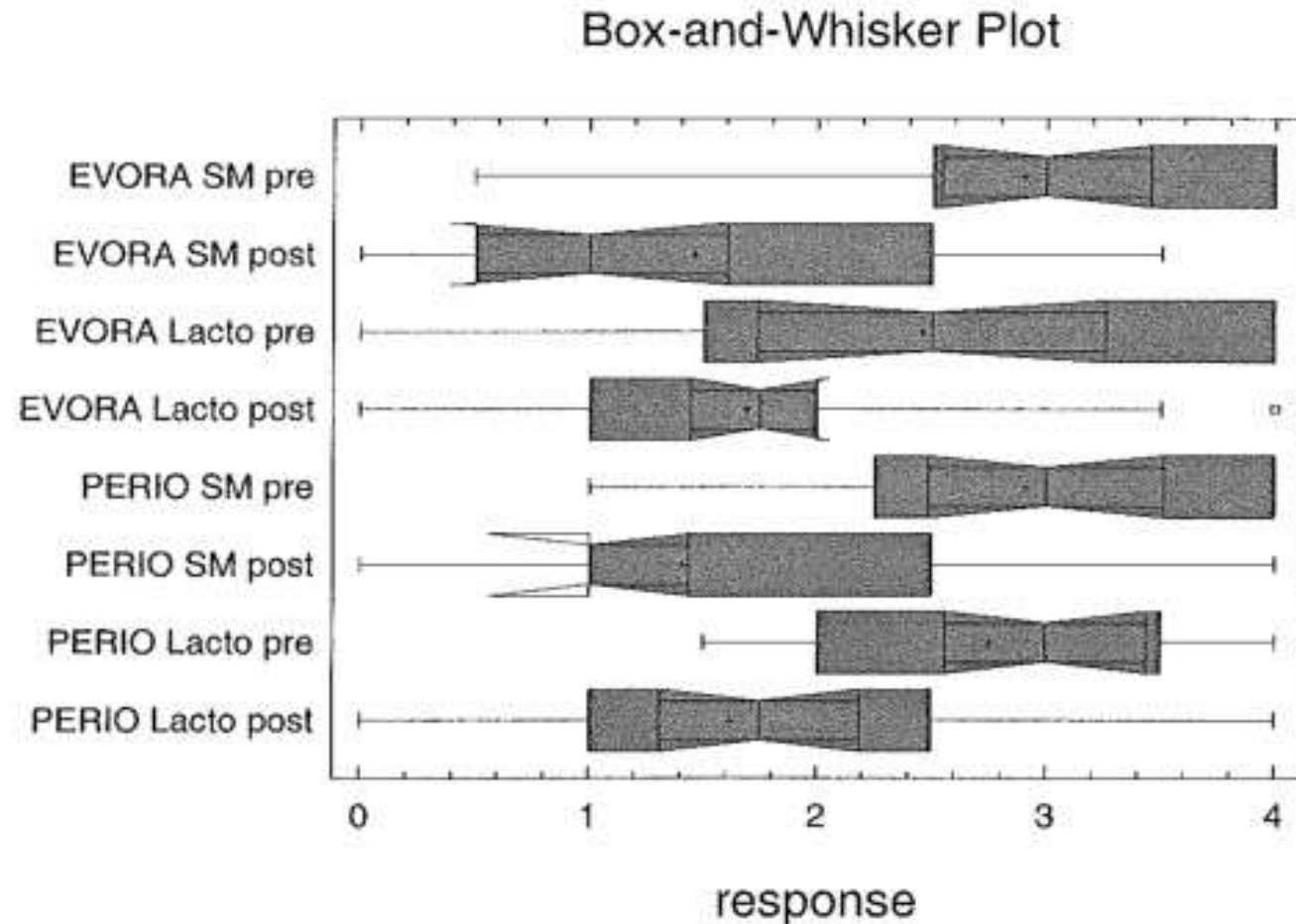




# DNA-PCR and CRT Results in Children After Probiotic use

## Statistics

Two separate statisticians in different institutions





# Conclusions:

Effectiveness of CRT at Measuring the Salivary Level of Bacteria in Caries Prone Children

## Effectiveness of CRT at Measuring the Salivary Level of Bacteria in Caries Prone Children with Probiotic Therapy

Cannon M\* / Trent B\*\* / Vorachek A\*\*\* / Kramer S\*\*\*\* / Esterly R\*\*\*\*\*

**Aim:** This IRB approved clinical trial was to determine the effect of "over the counter" probiotic supplements on the Caries Risk Test- CRT- (Proclar) results of the oral microflora in high caries risk children. **Study design:** Sixty subjects 6 to 12 years old with a caries risk assessment (CAMBRA) of moderate to high (caries prone) were evaluated by an analysis of the difference in the salivary levels of pathogenic bacteria (*mutans streptococci* and *Lactobacilli*). The subjects were randomly selected by randomizing software and assigned to two different Groups. Group A used PerioBalance (*Lactobacilli reuteri*-CFU of 200 million) lozenges for 28 days. Group B used the EvoraKids (*Streptococcus uberis* KJ2, *Streptococcus oralis* KJ3, *Streptococcus rattus* JH145,  $\geq 100$  million) probiotics chewable tablets for 30 days. Salivary samples were collected then incubated for 48 hours for colony counting and ranking. Follow up testing with the CRT was performed after 60 days at a follow up visit. **Results:** There was a statistically significant difference in the CRT results between the pre and post use of the probiotics. PerioBalance; SM results  $t = -6.78$ ,  $p < .0001$  Lactobacilli results  $t = -5.762$ ,  $p < .0001$ , EvoraKids SM results  $t = -7.33$ ,  $p < .0001$ , Lactobacilli results  $t = -2.952$ ,  $p = .0068$ . **Conclusions:** The CRT values obtained with caries prone children may be significantly affected by probiotic use. Based on this study's results the following conclusions can be made: Both EvoraKids and PerioBalance affected the CRT results by significantly decreasing the number of *S. mutans* and *lactobacilli* present in the salivary samples.

ed

acilli



# Further Research

**Retrospective  
Review of  
Probiotic  
Therapy.**

**ML Cannon DDS  
MS**

**A Vorachek DDS**

**K White DMD**

**C Le DMD**

**An IRB Approved  
Study**

Does EvoraKids and  
PerioBalance affected  
the caries proneness  
of the subjects?  
Is the reduction in  
*dental caries* was  
statistically  
significant?





# Further Research

## Materials and Methods:

Dental records of 60 patients that were

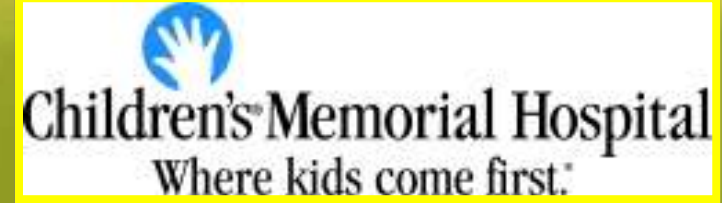
## Results:

Of the **53 subjects** available for follow up, **only 4 had remained caries active** with a grand total of 17 caries lesions being detected and subsequently restored in this group. Of the original group of caries active patients, 23 did not present with any further carious involvement. **Another 26 could be categorized as Caries static,** as the restorations required were substantially less than before probiotic therapy had been begun.

and then analyzed in respect to published national norms.



# Further Research



**Conclusion:**  
Within the limitations of this retrospective IRB approved study, the tested probiotic supplements had a statistically significant effect on the caries experience of the enrolled subjects.

**Table 3. Caries History Compared to Nationally Reported Values.**

Caries Experience	Pre Probiotic	National Average	Post Probiotic
Per patient- 3 years	5.51	1.84	0.75

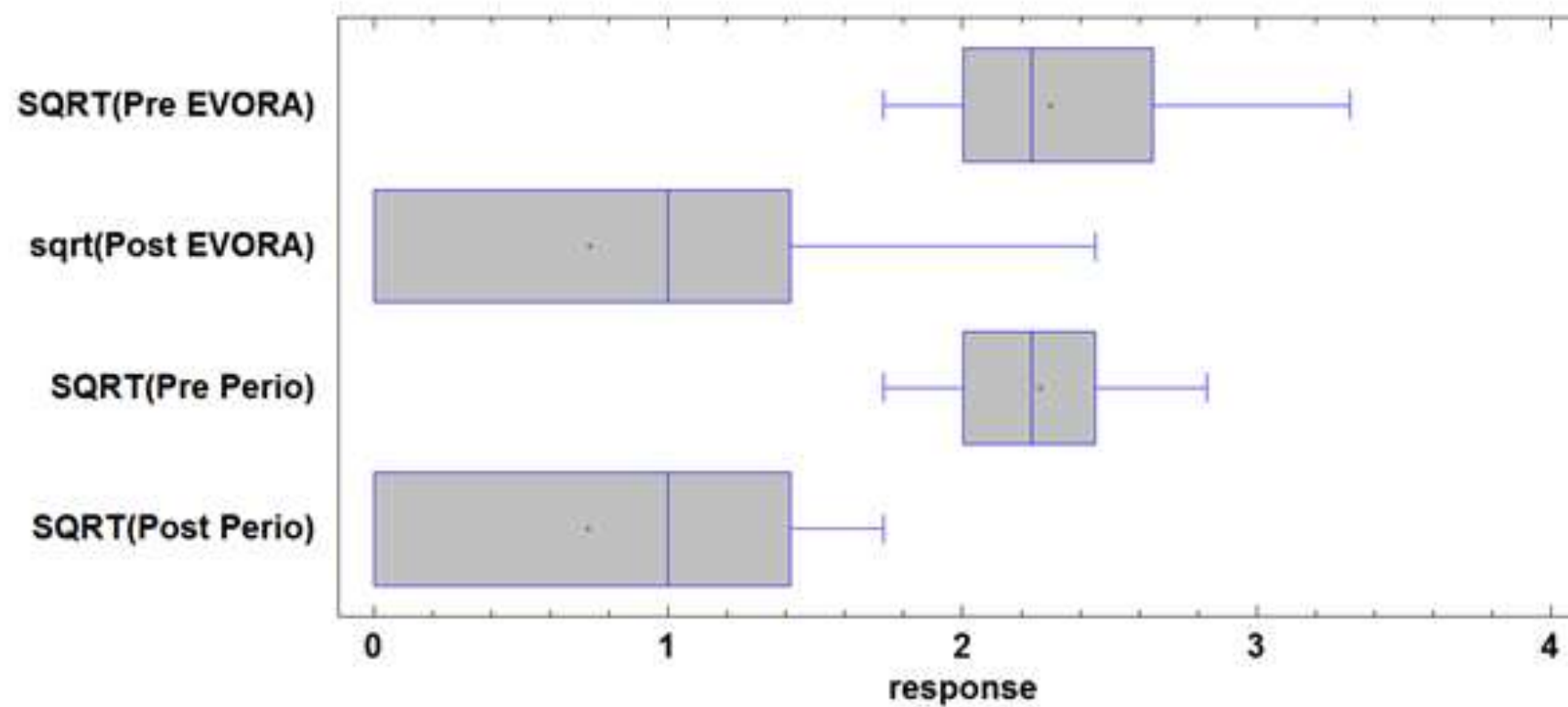
	Caries Active	Caries Resistant	Caries Static
PerioBalance	2	12	15
EvoraKids	2	11	11
Caries Count	17	0	36

**Table 1. Caries active, Caries resistant and Caries static patients.**



# Statistics

Box-and-Whisker Plot





The ANOVA table decomposes the variance of the data into two components: a between-group component and a within-group component. **The F-ratio, which in this case equals 51.3313, is a ratio of the between-group estimate to the within-group estimate.** The p-value is less than 0.001, indicating a significant difference between the groups. The confidence interval for the difference in means is [0.15, 0.35].



**Conclusion:** Within the limits of the study, the test results show a significant effect of the intervention on the subjects.



# Validation Study ( I told you so!)

Research article

Open Access

## Effect of probiotic chewing tablets on early childhood caries – a randomized controlled trial

Trifa Hedayati-Hajikand<sup>1,2</sup>, Ulrika Lundberg<sup>1</sup>, Catarina Eldh<sup>1</sup> and Svante Twetman<sup>3</sup>\*

# Low caries rate To begin with!

BMC Oral Health 2015, 15:112

doi:10.1186/s12903-015-0096-5



### Results

0.2 versus 0.8 cavity rate-

The groups were balanced at baseline and the attrition rate was 20 %. Around 2/3 of the children in both groups reported an acceptable compliance. The caries increment ( $\Delta$ ds) was significantly lower in the test group when compared with the placebo group, 0.2 vs. 0.8 ( $p < 0.05$ ). The risk reduction was 0.47 (95 % CI 0.24–0.98) and the number needed to treat close to five. No differences were displayed between the groups concerning presence of visible plaque or bleeding-on-brushing. No side effects were reported.



# Oral Health Probiotics- what to use?

- Probiora Pro
- Biogaia
- ProlacSan
- BLIS K12
- Prodegin
- Gluten metabolizers







**A healthy body  
starts with a  
healthy mouth.**



**ProBiora Health™**

Bringing  
the science  
of probiotics  
to oral care™

**ProBiōraPro™**



# Mission

“Bringing the science of probiotics to Oral Care”



About ProBiora Health™

ProBiora Health™ is bringing the science of probiotics to oral care by developing and marketing a complete line of proprietary probiotics that are specifically designed to enhance oral health for humans and pets. **Our products are based on ProBiora3® which was discovered by Dr. Jeffrey Hillman on the concept of replacement therapy.** **ProBiora3® is a blend of three naturally occurring strains of beneficial bacteria, including Streptococcus oralis KJ3, Streptococcus uberis KJ2, and Streptococcus rattus JH145, which support overall oral health.**



# ProBioraPro Product Overview



- Distributed exclusively by dental professionals
- Extra-strength blend of ProBiora3<sup>®</sup> crowds out harmful bacteria around teeth and gums
- Use once daily for 90 days after a dental hygiene visit
- Extends the benefits of a dental hygiene visit







## ProBiora3

- ProBiora3 is the most comprehensive oral care probiotic technology available
- Developed from research into dental caries and periodontal disease
- Blend of 3 naturally occurring *Streptococcal* strains – *S. oralis*, *S. uberis*, *S. rattus*
- ProBiora3 promotes:
  - dental and periodontal health
  - whiter teeth
  - fresher breath

## ProBiora3 Marketed as Food Ingredient

- Self-affirmed GRAS (Generally Recognized as Safe) status
- ProBiora3 is safe and effective
  - 15 peer-reviewed publications
  - Numerous peer-written periodicals
  - [www.probiorapro.com](http://www.probiorapro.com)





The ProBiora<sup>3</sup> contains a formulation of beneficial bacteria, found in naturally healthy human mouths:

***S. oralis* KJ3®**

***S. uberis* KJ2®**

***S. rattus* JH145®**

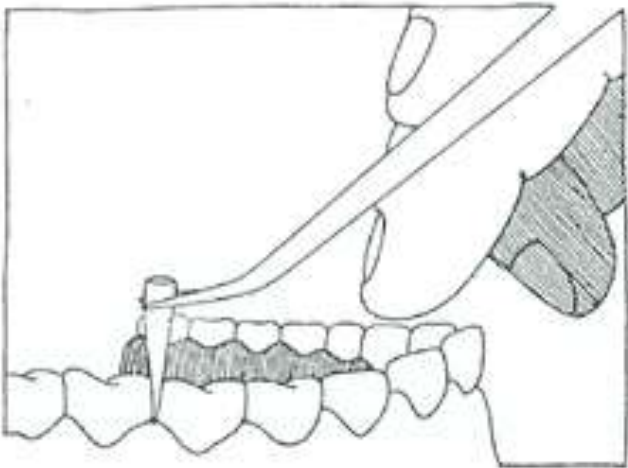
*S.rattus JH145, the third probiotic in ProBioraPro, is a unique strain of streptococcus that does not produce lactic acid, and has been shown to successfully compete for nutrients and space on tooth surfaces with the native strain of streptococcus that produces lactic acid. A study shows that 84% of people using ProBioraPro for a month experienced a decrease in the levels of S. mutans.*

*(A spontaneous lactate dehydrogenase deficient mutant of Streptococcus rattus for use as a probiotic in the prevention of dental caries. Hillman JD, McDonnell E, Cramm T, Hillman CH, Zahradnik RT. Journal of Applied Microbiology 2009 Nov;107(5):1551-8. Epub 2009 Apr 24)*

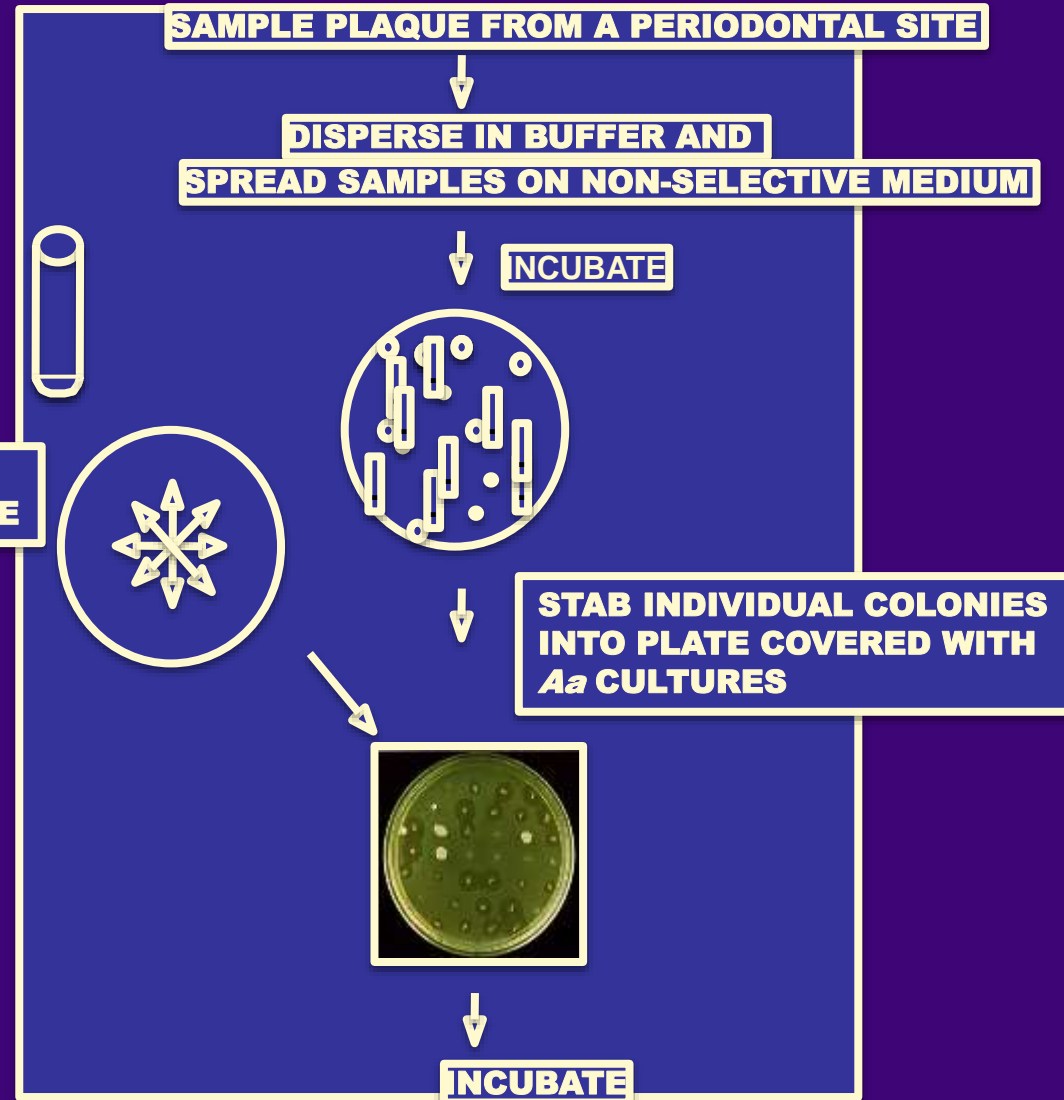


# Experimental Design

2.



**CROSS-STREAK SAMPLE OF  
*Aa* CULTURE ON FRESH PLATE**

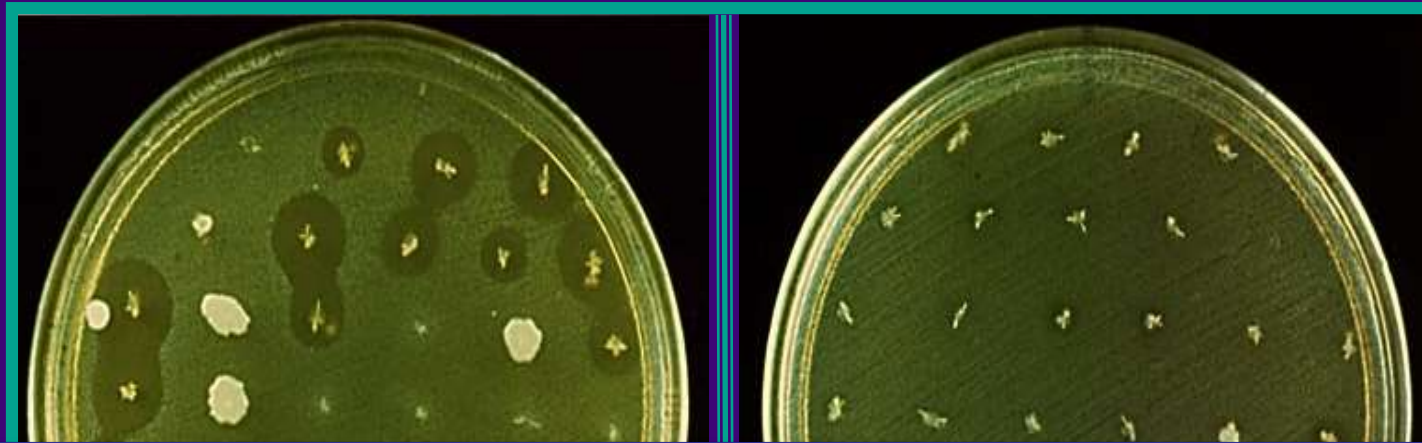






Healthy

Diseased

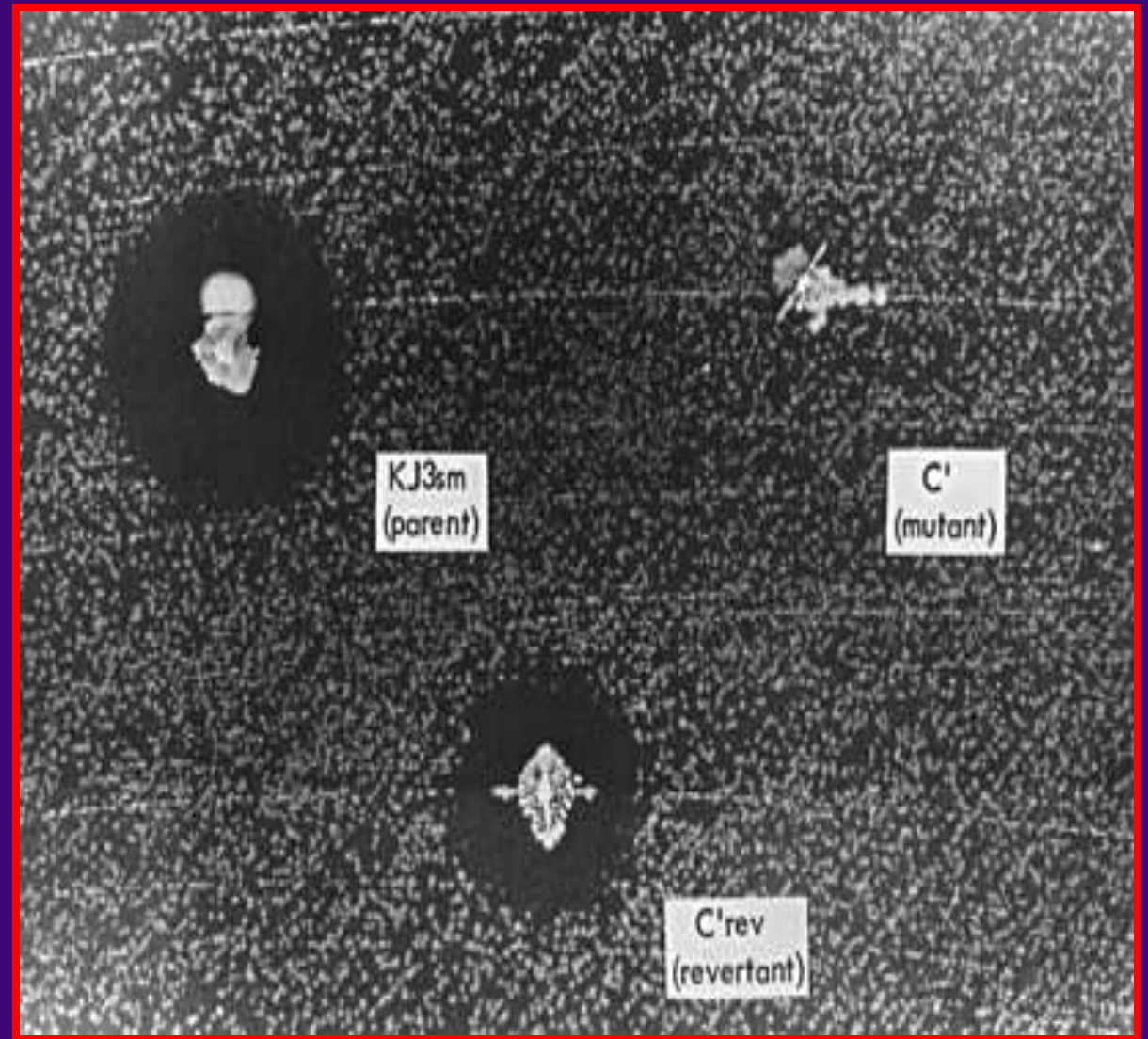


What creates a  
healthy mouth are the commensal  
bacteria!!!!



# Basis for the Negative Interaction

- The mechanism of inhibition by *S. oralis* is production of hydrogen peroxide.
- Research that this interaction occurs in a rat model.





Socransky, S.S. and Haffajee, A.D. The periodontal disease: current concepts. 322-331, 1992.

Following conventional periodontal therapy, the long-term success of the therapy is dependent on the presence of *Streptococcus* *veridans*, including *S. o* periodontal sites.

Hillman, J.D. and Shivers, M. Interaction of revertant forms of the bacterium *Streptococcus* *actinobacillus actinomycet* *gnotobiotic rat*. Arch. Oral Biol. 33: 39.

*Streptococcus oralis* and *Streptococcus* inhibit the growth of periodontal pathogens. They produce significant amounts of hydrogen peroxide, which is a toxic agent to the pathogens.

Socransky, S.S., Haffajee, A.D., Dzink, J.L. and Hillman, J.D. Associations between microbial species in subgingival plaque samples. Oral Microbiol. Immunol. 3: 1-7, 1988.

The presence of *Streptococcus oralis* and *Streptococcus uberis* was shown to reduce the risk of finding various periodontal pathogens in dental plaque. The relationship was shown to be dose-dependent by demonstrating that the risk of finding a particular pathogen decreased as the proportion of the beneficial strains increased.

Hillman, J.D., Socransky, S.S. and Shivers, M. The relationships between streptococcal species and periodontopathic bacteria in human dental plaque. Arch. Oral Biol. 30: 791-795, 1985.

Plaque from healthy subjects and from healthy sites in patients with periodontal (gum) disease was shown to contain bacteria that inhibit the growth of a certain bacteria known to cause periodontal disease. In contrast, plaque from diseased sites in subjects with periodontal disease was shown to lack these beneficial bacteria. The beneficial bacteria were identified as *Streptococcus oralis* and *Streptococcus uberis*.

Johnson, C.P., Gross, S.M. and Hillman, J.D. \*\* Cariogenic potential in vitro in man and in vivo in the rat of lactate dehydrogenase mutants of *Streptococcus mutans*. Arch. Oral Biol. 25: 707-713, 1980.

JH145, a completely natural strain of *Streptococcus rattus* - which until recently was considered to be one of several subspecies of *Streptococcus mutans* - was shown to make virtually no lactic acid. Since the strain does not make lactic acid, it was demonstrated to be essentially incapable of causing dental caries in a rat model.



SURF



# Take Home- probiotics



- Probiora has decades of research backing the product.
- Most products have no studies.....
- Biogaia has over 160 studies.





# Take Home- probiotics



- Must have a protocol in place and training of your team- Test, Prebiotic, Probiotic and a way of measuring success. (CamX Spectra and Cariscreen again) Clinical Results!





Take H



- Prok
- “smo





**“Smoke without fire”**



**Waterloo  
June  
18<sup>th</sup>.,  
1815**

- Marshall Ney leads Cavalry charge against British squares, without infantry support- and fails to break their lines





# Lifeway Kefir-



Kefir is loaded with **tryptophan**, the amino acid that helps raise the levels of serotonin in your brain

- Lifeway Kefir is a tart and tangy cultured milk smoothie that is high in protein, calcium and vitamin D. Due to their exclusive blend of kefir cultures, each cup of kefir contains **12 live and active cultures and 15 to 20 billion beneficial CFUs.**



# Lifeway Kefir- ProBugs



- Frozen kefir for kids
- Great dessert item or health treat after a lot of outside play



# Skin Probiotics- Barrier!



• USDA Certified Organic - to assure quality and safety

• Made in the USA to produce the best quality of product available

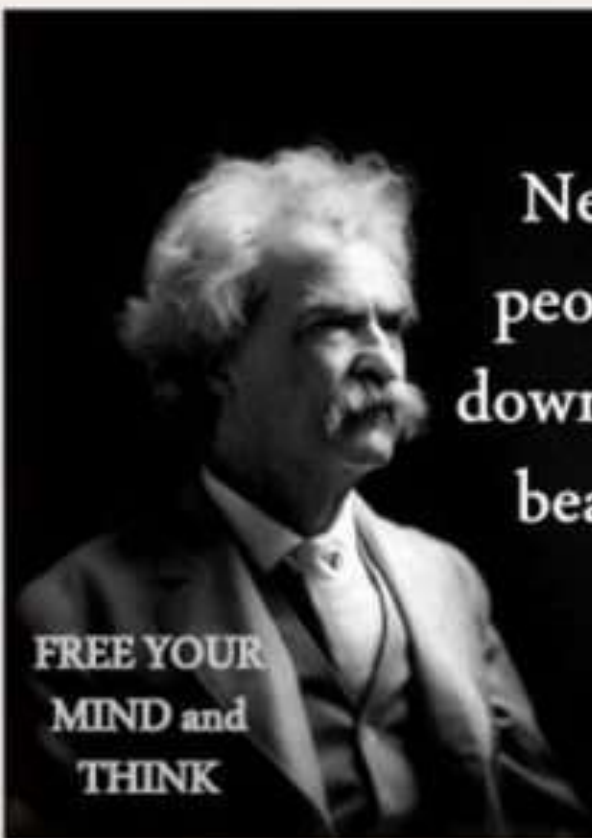
• Multi-Strains includes: **Lactobacillus Acidophilus, L. Rhamnosus, L. Salivarius, L. Rhamnosus, L. Casei, L. Plantarum, Lactococcus Lactis and Casei; Bifidobacterium Infantis, Longum along with Streptococcus Thermophilus all in a enzyme enriched substrate.**

• Ingredients: Derived from a proprietary blend of probiotics in an enzyme-enriched substrate, water and a proprietary blend of 3 organic grasses.





# Skin Probiotics- Barrier!



Never argue with stupid  
people, they will drag you  
down to their level and then  
beat you with experience.  
~Mark Twain

FREE YOUR  
MIND and  
THINK



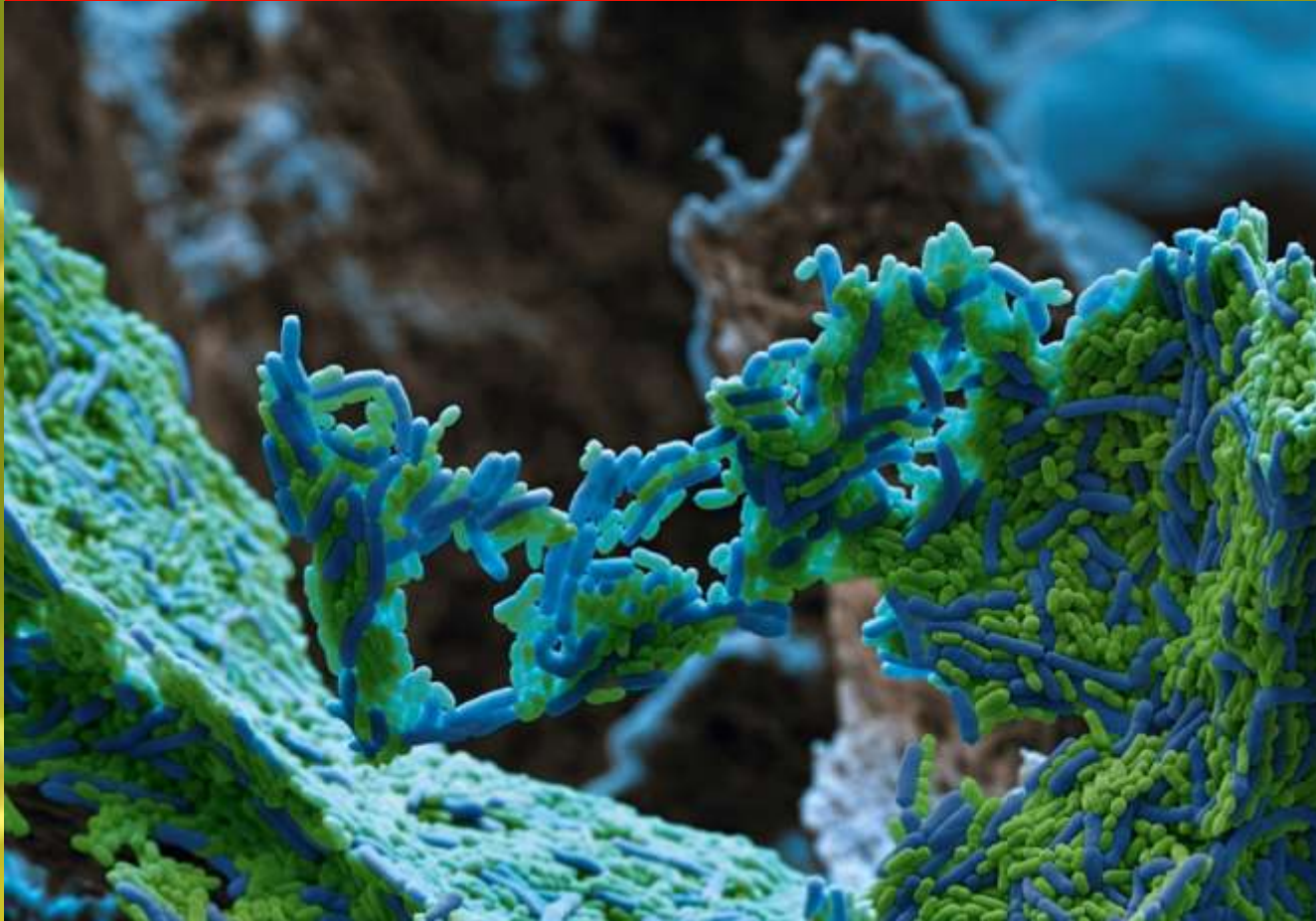
- So many products- SO expensive at Bloomingdales- no mention of which bacteria either- bundled with colloidal silver as “anti-bacterial” 75\$



# ***Lactobacilli paracasei***

pasteurized bacteria

BASF set to commercialize pro-  
t-action™ eliminating caries  
causing bacteria from the mouth







VOLUME 89



## RESEARCH Biological

C. Lang<sup>1\*</sup>,  
M. Veen<sup>1</sup>,  
M. Pompei<sup>2</sup>

<sup>1</sup>ORGANOBA  
D-13355 · Ber  
GmbH, 4 Garte  
and <sup>2</sup>University  
CT 06030-160  
organobalance.s

J Dent Res 89)

### ABSTRACT

Selective inte  
benign bacteri  
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oral flora. Thi  
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egy for in vivo

**KEY WORDS**  
streptococci,

DOI: 10.1177/

Received May  
Accepted Sept





- Going too far!

## Preventive Care



# Probiotics? Some caution necessary!

Pediatrics.

**Lactob**

Land MH,

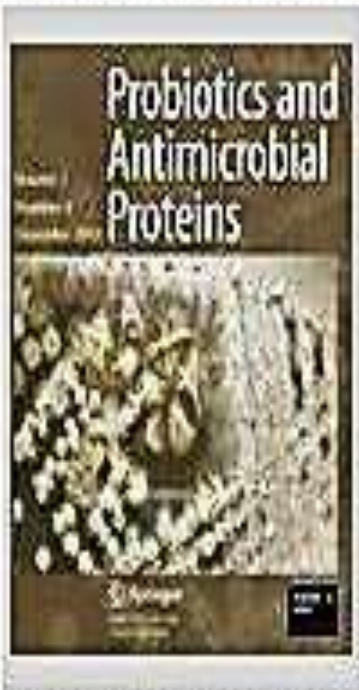
Department

**Abstract**

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## PROBIOTICS AND ANTIMICROBIAL PROTEINS

Volume 3, Number 2, 63-67, DOI: 10.1007/s12602-011-9072-9



## A Review of Probiotic Therapy in Preventive Dental Practice

Mark L. Cannon

• W

Requires understanding.

?



# Probiotics often forgotten

## Bacillus subtilis

**JADA**  
The Journal of the American Dental Association

PROBIOTIC & BACILLUS  
SUBTILIS







## Probiotics- but first prebiotics

- **PREBIOTIC FIBER** is a non-digestible component of foods like bananas, onions and garlic, Jerusalem artichoke, the skin of apples, chicory root, beans, and many others. Prebiotic fiber goes through the small intestine undigested and is fermented when it reaches the large colon.



# Prebiotics!!

**What are prebiotics?**

**Prebiotics are selectively fermented ingredients that allows specific changes, both in the composition and/or activity in the gastrointestinal microflora that confers benefits upon host well-being and health (Roberfroid 2007).**

**Oligosaccharides**





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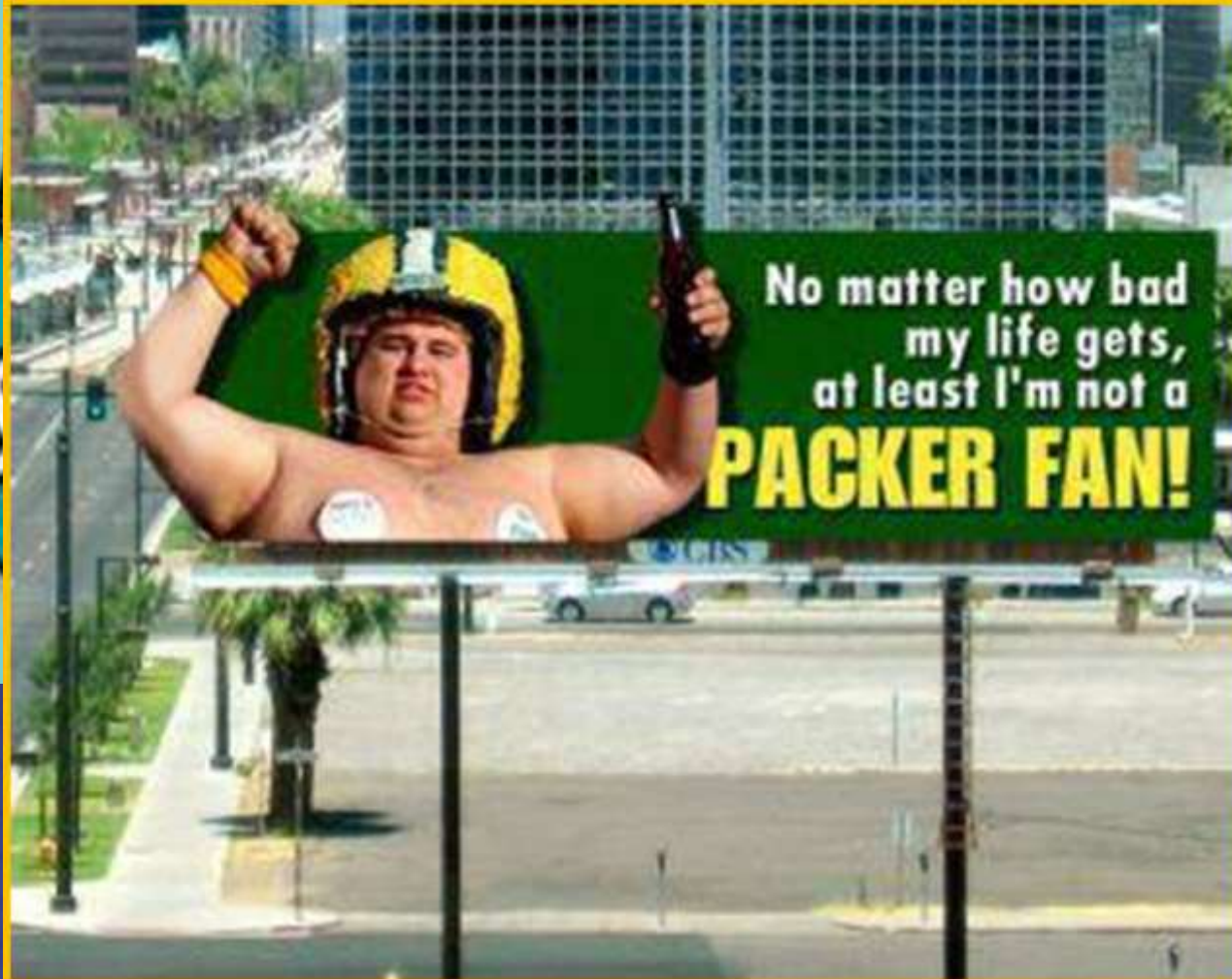
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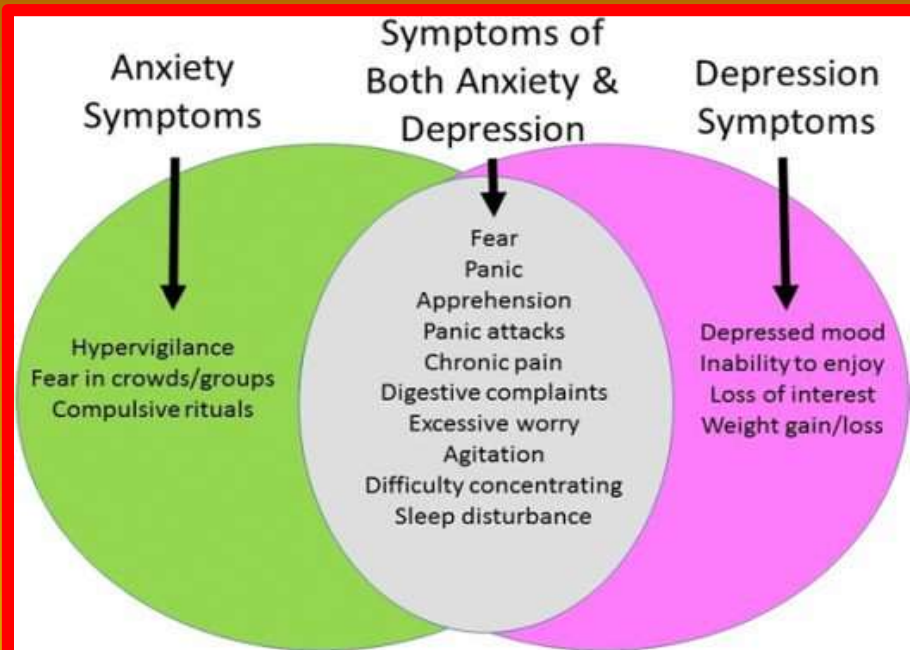
mlc





# Probiotics and Microbiome

## • Anxiety Depression







# Neurologic

## Probiotics- kefir



Gastroenterology. 2013 Jun;144(7):1394-401, 1401.e1-4. doi: 10.1053/j.gastro.2013.02.043. Epub 2013 Mar 6.

### Consumption of fermented milk product with probiotic modulates brain activity.

Tillisch K<sup>1</sup>, Labus J, Kilpatrick L, Jiang Z, Stains J, Ebrat B, Guyonnet D, Legrain-Raspaud S, Trotin B, Naliboff B, Mayer EA.

#### Author information

#### Abstract

##### BACKG

reflexes  
humans  
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Multivari

##### RESULT

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FMPP was associated with changes in midbrain connectivity, which could explain the observed differences in activity during the task.

**CONCLUSIONS:** Four-week intake of an FMPP by healthy women affected activity of brain regions that control central processing of emotion and sensation.

# Functional MRI and emotional brain tasking

FMPP changed mid brain connectivity- responses to emotional attention tasks



# Probiotics- Treatment of Depression

Brain Behav Immun. 2015 Apr 7. pii: S0926-6410(15)00100-0.

## A randomized controlled trial of the effect of a probiotic on cognitive reactivity in major depression

Steenbergen L<sup>1</sup>, Sellaro R<sup>2</sup>, van den Heuvel PA, et al.

### Author information

#### Abstract

**BACKGROUND:** Recent insights into the gut-brain axis suggest that probiotic supplementation may act as a modulator of mood and cognition.

**OBJECTIVE:** Heightened cognitive reactivity to sad mood is considered an important target in the treatment of major depression. Bifidobacterium lactis W52, Lactobacillus acidophilus W81, and Lactococcus lactis (W19 and W22) are probiotics that have been shown to have beneficial effects on mood and cognition.

**DESIGN:** In a triple-blind, placebo-controlled, randomized trial, 40 patients with major depression received a 4-week probiotic or placebo for the same period. The primary outcome was the index of depression sensitivity to sad mood.

**RESULTS:** Compared to placebo, the probiotic group showed a significantly reduced index of depression sensitivity to sad mood.

**CONCLUSION:** These results suggest that probiotic supplementation may be a useful adjunct in the treatment of major depression.



print]

otics on cognitive reactivity

strains- multi-species

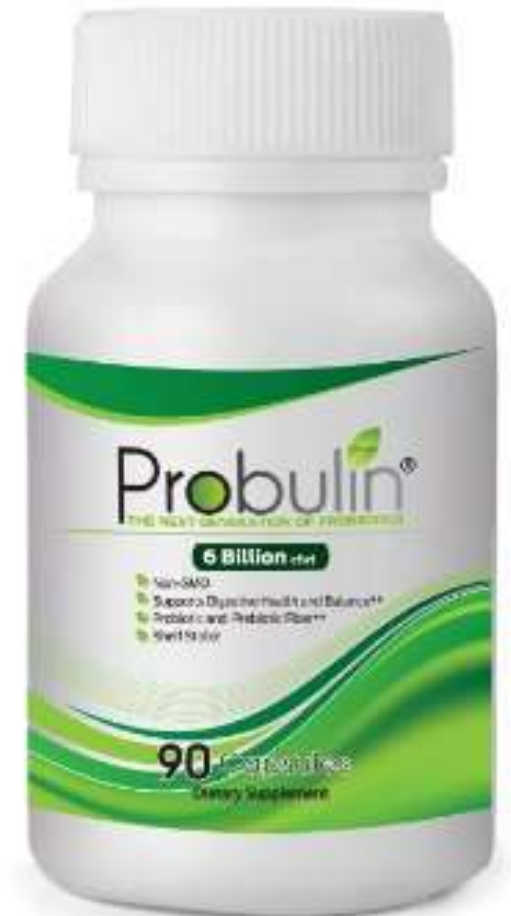
ective functioning have led to

n established marker of vulnerability. A multi-species probiotic containing Lactobacillus casei W56, Lactobacillus acidophilus W81, and Lactococcus lactis (W19 and W22) was evaluated.

sment design, 20 healthy participants received the probiotic, while 20 control participants received placebo. The primary outcome was the index of depression sensitivity to sad mood was assessed.

o received the 4-week multi-species probiotic. The primary outcome was the index of depression sensitivity to sad mood was assessed.

help reduce negative thoughts associated with sad mood. The results suggest that probiotic supplementation may be a useful adjunct in the treatment of depression.





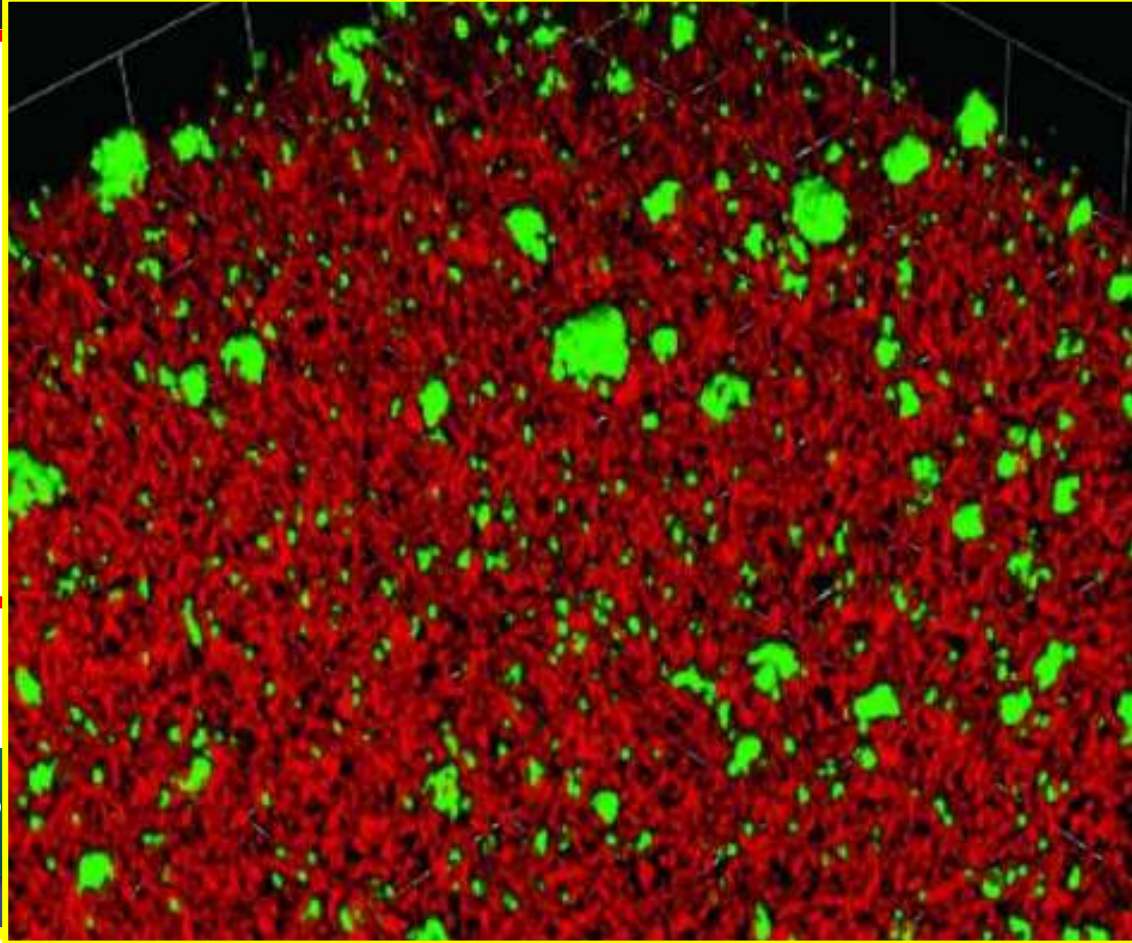


# Special Needs Patients

- **Emphasis on:**
  - **Autism Spectrum Disorder**
  - **Alzheimer's Disease**
  - **Anxiety Disorders**
  - **Allergies**
  - **Airway (SDB)**



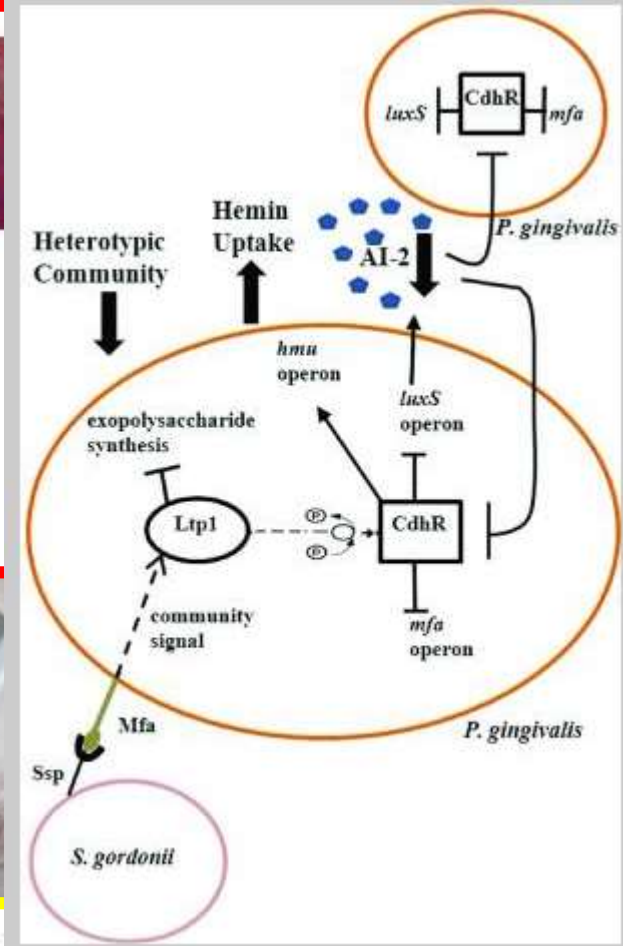
# Probiotics- Neurologic Implications



virulence

P), which is key to

zyme



High levels of STEP proteins keep synapses in the brain from strengthening. Synaptic strengthening is a process that is required for people to turn short-term memories into long-term memories. When STEP is elevated in the brain, it depletes receptors from synaptic sites, and inactivates other proteins that are necessary for proper cognitive function. This disruption can result in Alzheimer's disease or a number of neuropsychiatric and neurodegenerative disorders, all marked by cognitive deficits.



# Alzheimer's Diseases- Three types

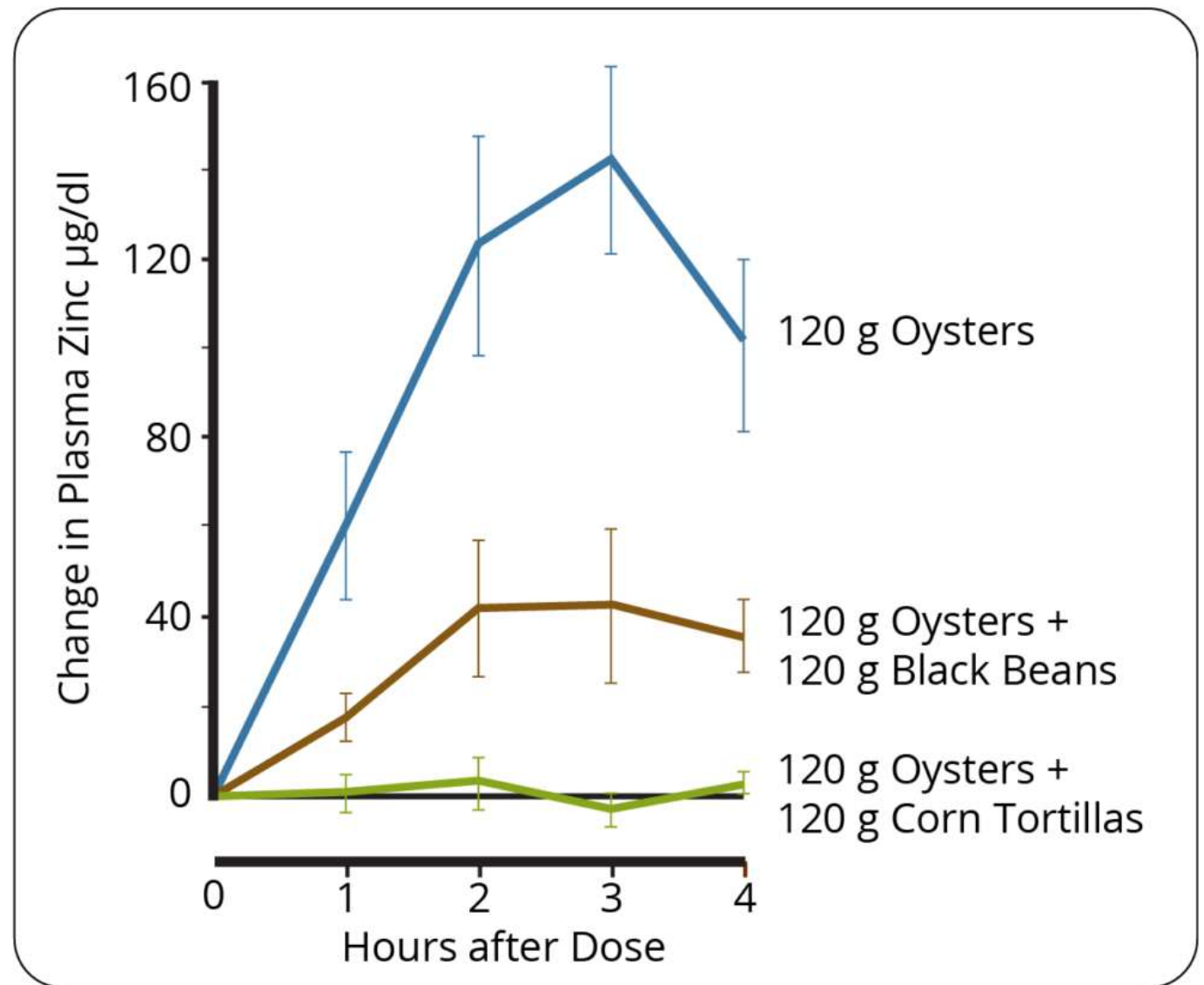
Alzheimer's disease consists of three types

Date: September 16, 2015

Source: University of California, Los Angeles

Summary: Alzheimer's disease, long thought to be a common age-related dementia, and is expected to increase to 15 million in 2050, from 5 million in 2010.

The subtypes are: • **Inflammatory**, which affects relatively young individuals. The brain than the other subtypes of Alzheimer's disease. It is often misdiagnosed as Alzheimer's-related gene and is associated with





# ED and Dementia- Probiotics

Life is cruel and unfair, my  
friends, and that is fact.

Stephan Jenkins

quote fancy

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After ad  
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patients





# Nutrition and Autism

Adams et al. *Nutrition & Metabolism* 2011, **8**:34  
<http://www.nutritionandmetabolism.com/content/8/1/34>



**Nutrition & Metabolism**

RESEARCH

Open Access

## Nutritional and metabolic status of children with autism vs. neurotypical children, and the association with autism severity

James B Adams<sup>1\*</sup>, Tapan Audhya<sup>2</sup>, Sharon McDonough-Means<sup>3</sup>, Robert A Rubin<sup>4</sup>, David Quig<sup>5</sup>, Elizabeth Geis<sup>1</sup>, Eva Gehn<sup>1</sup>, Melissa Loresto<sup>1</sup>, Jessica Mitchell<sup>6</sup>, Sharon Atwood<sup>1</sup>, Suzanne Barnhouse<sup>1</sup> and Wondra Lee<sup>1</sup>

The autism group had many statistically significant differences in their nutritional and metabolic status, including biomarkers indicative of vitamin insufficiency, increased oxidative stress, reduced capacity for energy transport, sulfation and detoxification. Several of the biomarker groups were significantly associated with variations in the severity of autism.



# Probiotics and Microbiome

- **Autism  
Spectrum  
Disorder**





# Special Care for Special Patients



**CDC estimate of autism prevalence increases to 15%. Now 1 in 59 children.**

**Autism Speaks calls on nations' leaders to adequately fund critical research and resources**

**NEW YORK (April 26, 2018)** The Centers for Disease Control and Prevention (CDC) today released its biennial update of autism's estimated prevalence among the nation's children, based on an analysis of 2014 medical and/or school records of 8-year-olds from 11 monitoring sites across the United States. The report demonstrates that while progress has been made on some fronts, there is still critical work to do.

**restricted and repetitive behavior.**

Repetitively stacking or lining up objects is a behavior occasionally associated with individuals with autism.





# Special Care f

- The diagnostic cr  
symptoms becom  
before a child is t

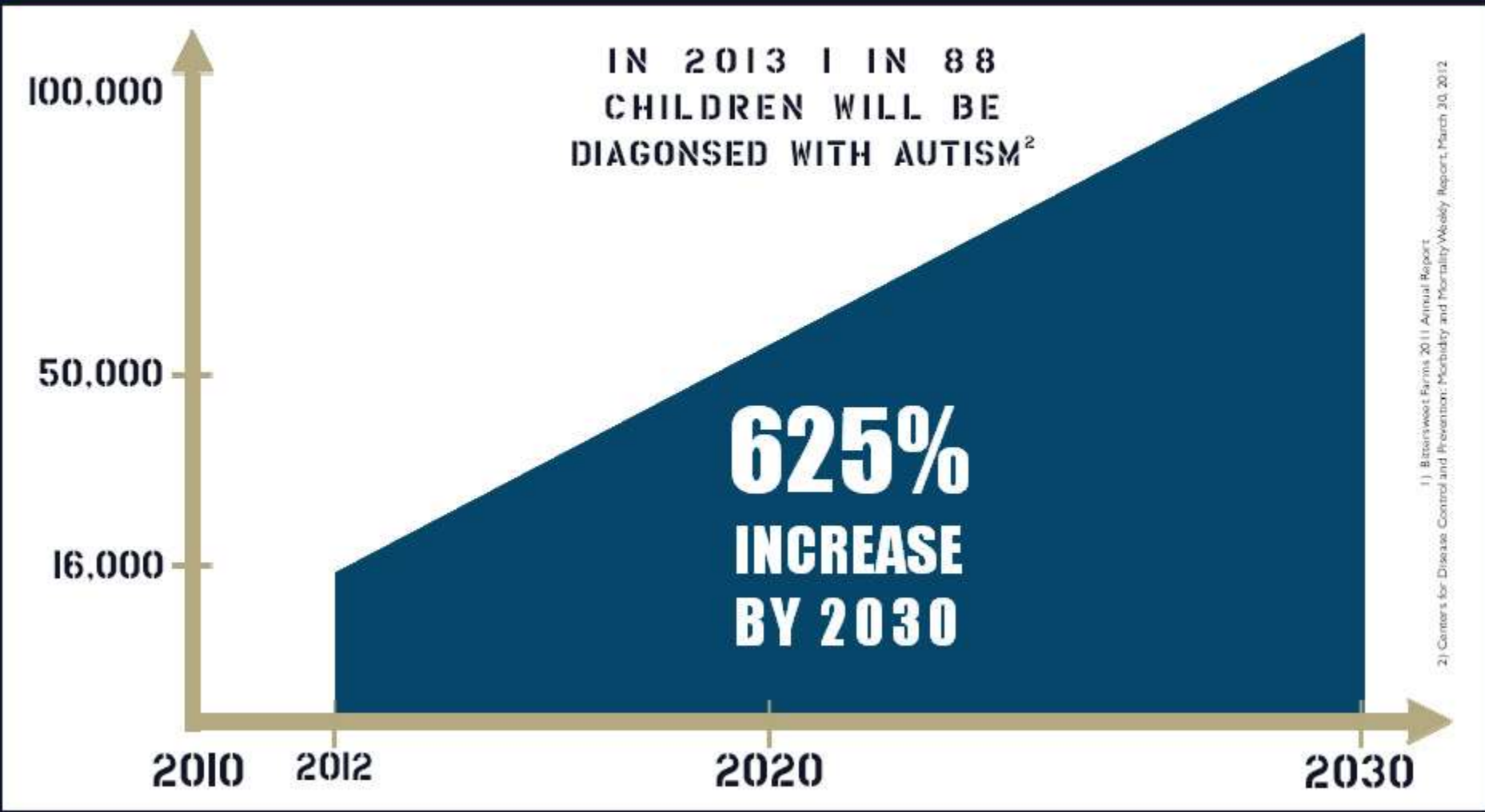
A young boy w  
autism who has  
arranged his to  
order of size





# Probiotics- Neurologic Implications

**AUTISM WILL INCREASE BY 625% BY 2030<sup>1</sup>**



Autism Model



## autism spectrum

ASD and MD do not have children with ASD. A product of behavioral, metabolic, pattern of elevations in biomarkers of ASD (n=213) who d autism clinic were rmal in the panel and rmal acyl-carnitine panels.

of autism shows hyperactivity and repetitive of brain tissue (hippocampus) from autism (increased staining density) in glia and brain) similar to tissue obtained from brains from MacFabe et al, 2007, Behavioural Brain

as a valid animal model of the condition. Collectively, this offers further supp such as dietary or enteric bacterially produced SCFAs, may be plausible environmental agents that can trigger ASDs or ASD-related behaviors and deserve further exploration in basic science, agriculture, and clinical medicine.



# Autism- and other dilemmas

Microbial Ecology in Health  
and Disease



Taylor & Francis  
Taylor & Francis Group

*Microb Ecol Health Dis.* 2015; 26: 10.3402/mehd.v26.26914.  
Published online 2015 Mar 12. doi: [10.3402/mehd.v26.26914](https://doi.org/10.3402/mehd.v26.26914)

PMCID: PMC4359272

## **Gut bacteria in children with autism spectrum disorders: challenges and promise of studying how a complex community influences a complex disease**

[Rosa Krajmalnik-Brown](#),<sup>1,2,\*</sup> [Catherine Lozupone](#),<sup>3</sup> [Dae-Wook Kang](#),<sup>1</sup> and [James B. Adams](#)<sup>4</sup>

Here we first summarize previously published data supporting that **GI dysfunction** is common in individuals with ASD and the role of the **microbiota in ASD**. Second, by comparing with other publically available microbiome datasets, we provide some evidence that the shifted microbiota can be a result of westernization and that this shift could also be framing an **altered immune system**. Third, we explore the possibility that gut–brain interactions could also be a direct result of microbially produced metabolites.



# Autism- and other dilemmas

Microbial Ecology in Health  
and Disease



Taylor & Francis  
Taylor & Francis Group

*Microb Ecol Health Dis.* 2015; 26: 10.3402/mehd.v26.26914.

PMCID: PMC4359272

Published online 2015 Mar 12. doi: [10.3402/mehd.v26.26914](https://doi.org/10.3402/mehd.v26.26914)

## **Gut bacteria in children with autism spectrum disorders: challenges and promise of studying how a complex community influences a complex disease**

Rosa Kraïmalnik-Brown,<sup>1,2,\*</sup> Catherine Lozupone,<sup>3</sup> Dae-Wook Kang,<sup>1</sup> and James B. Adams<sup>4</sup>

**Prevotella**, is highly enriched in the fecal microbiota in populations in Africa including agrarian societies in Malawi and Burkina Faso , and the Hadza hunter–gatherers in Tanzania intrigued us and inspired us to perform comparative analyses. Since **Prevotella** is only one genus in the very diverse gut microbiota and has a tendency to co-occur with a complex collection of other bacteria species, we wanted to determine whether **Prevotella depletion in children with ASD** is an indicator that the gut microbiome of children with ASD who live in the United States differs even more from individuals in the developing world than does the gut microbiome of neurotypical children in the US, providing evidence of the gut microbiota as an environmental factor that may correlate with increased rates of ASD in industrialized countries.



# Autism

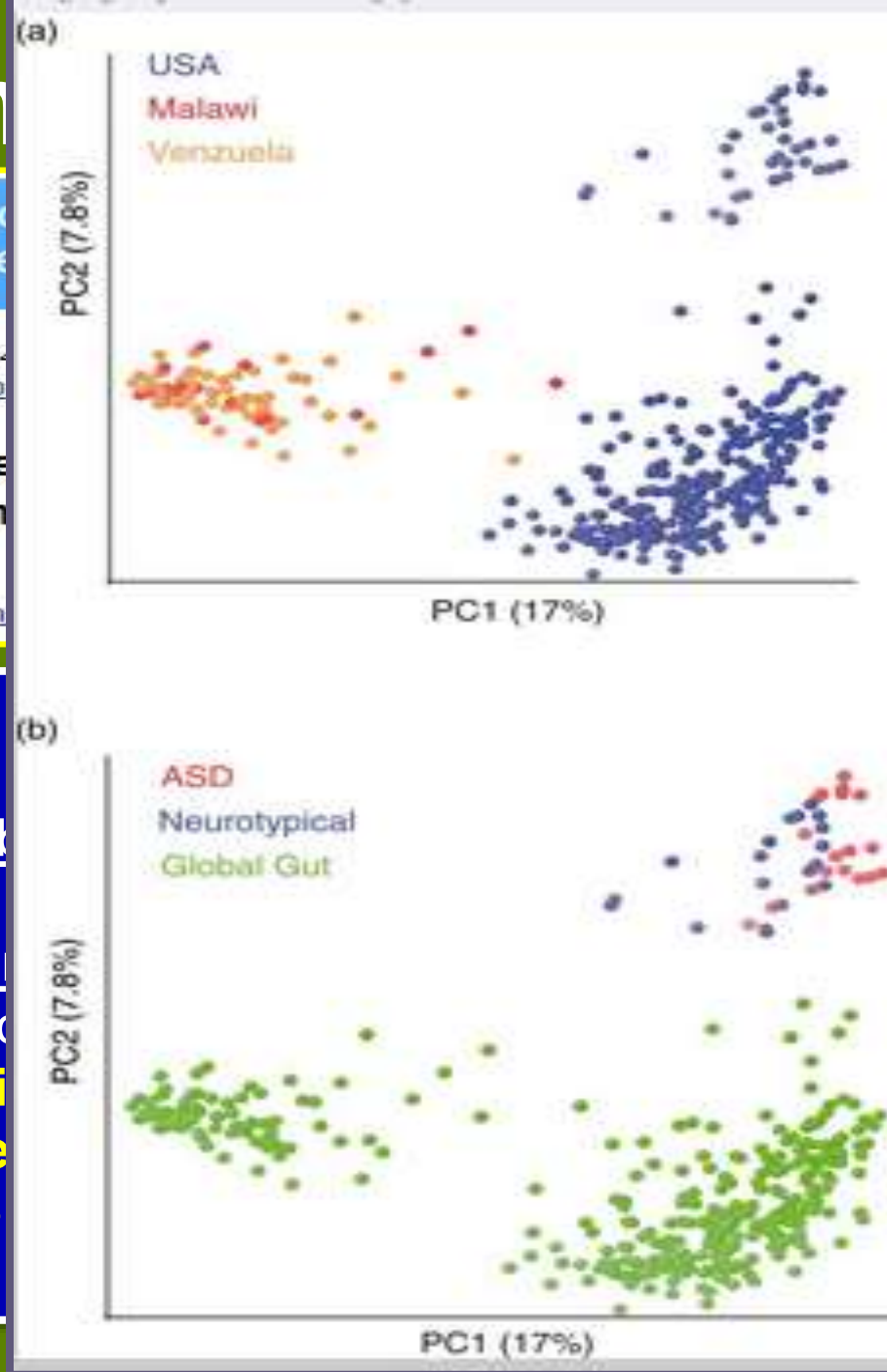
## Microbial Ecology and Disease

Microb Ecol Health Dis. 2015; 26: 10.34  
Published online 2015 Mar 12. doi: 10.

### Gut bacteria in children promise of studying h disease

Rosa Kraïmalnik-Brown,<sup>1,2,\*</sup> Cath

A high rate of GI problems severity and GI symptoms microbes and their metabolites a link between the gut and the potential for a role for symptoms. Furthermore, microbiota of children with that are driven by unique developing world lead to with ASD.



# mas



symptom  
es of gut  
ng appreciation of  
all point towards  
severity of ASD  
n' of the gut  
ta differences  
red to the  
a composition



# Autism Spectrum Disorders

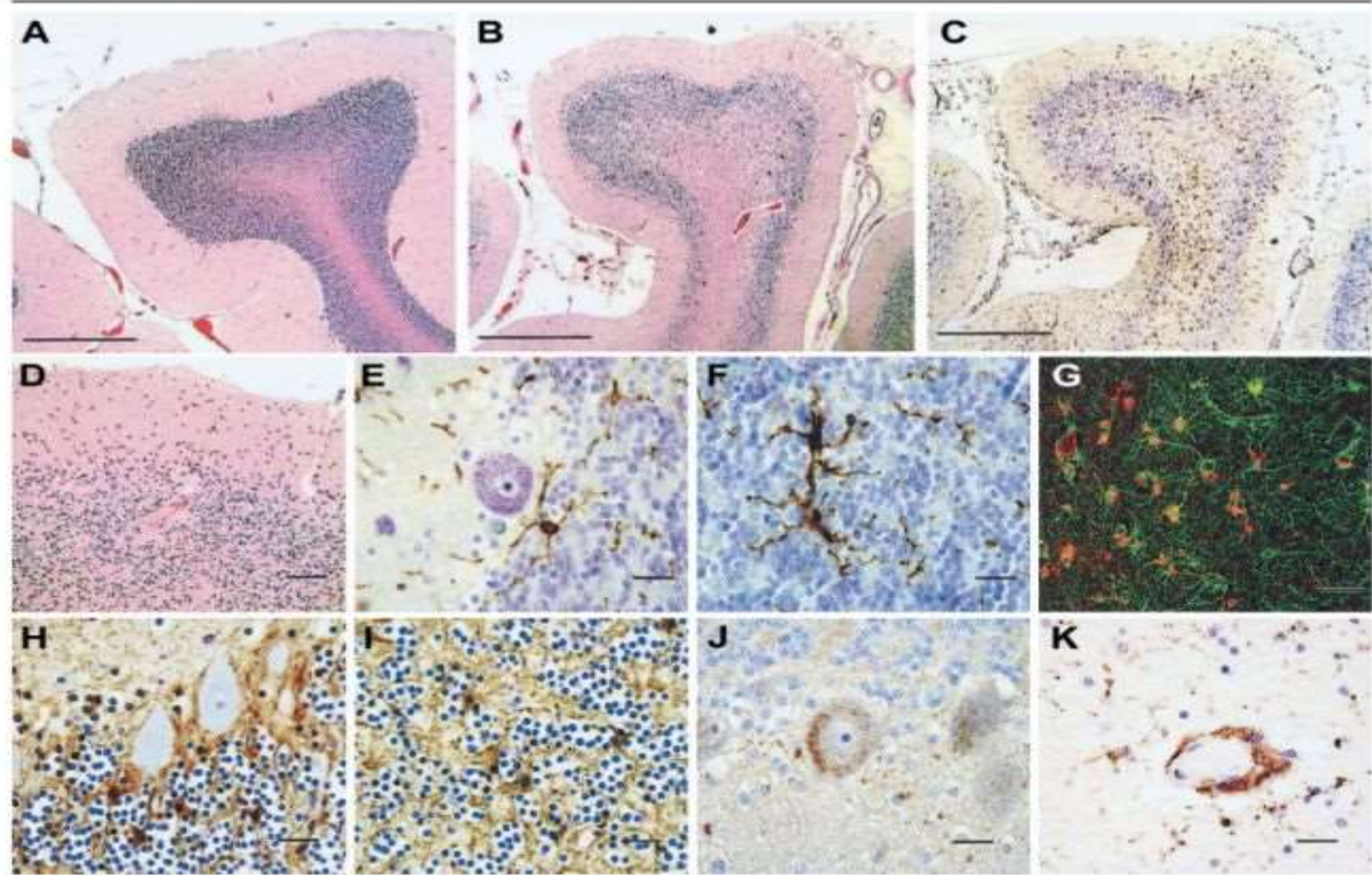
## Neuroglial activation in autism

Diana L. Vargas MD<sup>1,2</sup>,  
Nascimbene MD<sup>1,2,3</sup>,  
MHS<sup>1</sup>, Andrew W. Zimmerman MD<sup>1,2</sup>,  
and Carlos A. Pardo MD<sup>1,2</sup>

Article first published online May 1, 2003

DOI: 10.1002/ana.20311

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**Fig 1. Cerebellar pathology in autism.** (A) Normal cerebellar folia in a control brain (H and E staining). (B) Patchy loss of Purkinje cell layer (PCL) and granular cell layer (GCL) neurons (H and E) and (C) marked activation of microglia (immunostained with anti-HLA-DR antibody) are seen in the cerebellar folia of a patient with autism. Bar in A–C = 500µm. (D) High-magnification detail of a cerebellar region with marked PCL and GCL neuronal loss (H and E). Bar = 50µm. (E, F) Activated microglia around a Purkinje cell (E) and in the GCL (F), immunostained with anti-HLA-DR. Bar in E and F = 20 µm. (G) Close relationship of



# Probiotics- Neurologic Implications



Microbial fermentation contributes to disorders of propionate metabolism, control.

Specific treatment on propionate concentrations in a child with

study the effects of addition of (and vancomycin) on net faecal acid. Courses of oral antibiotics of 7 faecal propionate production and tions.

Consistent reduction (77–84%) in the s. Oral administration of 3% within 24 hours of treatment; a 7 se reductions were accompanied by during the same period.

It might be as effective as continuous n with disorders of propionate



# Autism Spectrum Disorders

## Valproate Model for Autism- tells all!



Neurosci Lett. 2010 Feb 5;470(1):55-9. doi: 10.1016/j.neulet.2009.12.054. Epub 2009 Dec 28.

### **Behavior and serotonergic disorders in rats exposed prenatally to valproate: a model for autism.**

Dufour-Rainfray D<sup>1</sup>, Vourc'h P, Le Guisquet AM, Garreau L, Ternant D, Bodard S, Jaumain E, Gulhan Z, Belzung C, Andres CR, Chalon S, Guilloteau D.

JAMA. 2013 Apr 24;309(16):1696-703. doi: 10.1001/jama.2013.2270.

### **Prenatal valproate exposure and risk of autism spectrum disorders and childhood autism.**

Christensen J<sup>1</sup>, Grønborg TK, Sørensen MJ, Schendel D, Parner ET, Pedersen LH, Vestergaard M.

Anat Rec (Hoboken). 2010 Nov;293(11):1947-53. doi: 10.1002/ar.21232.

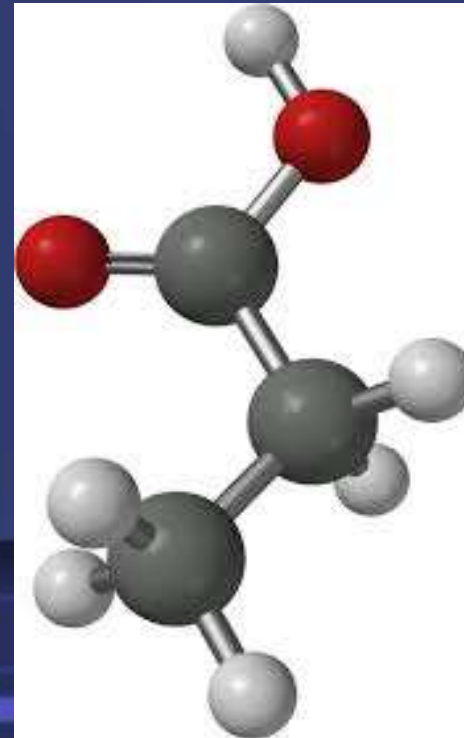
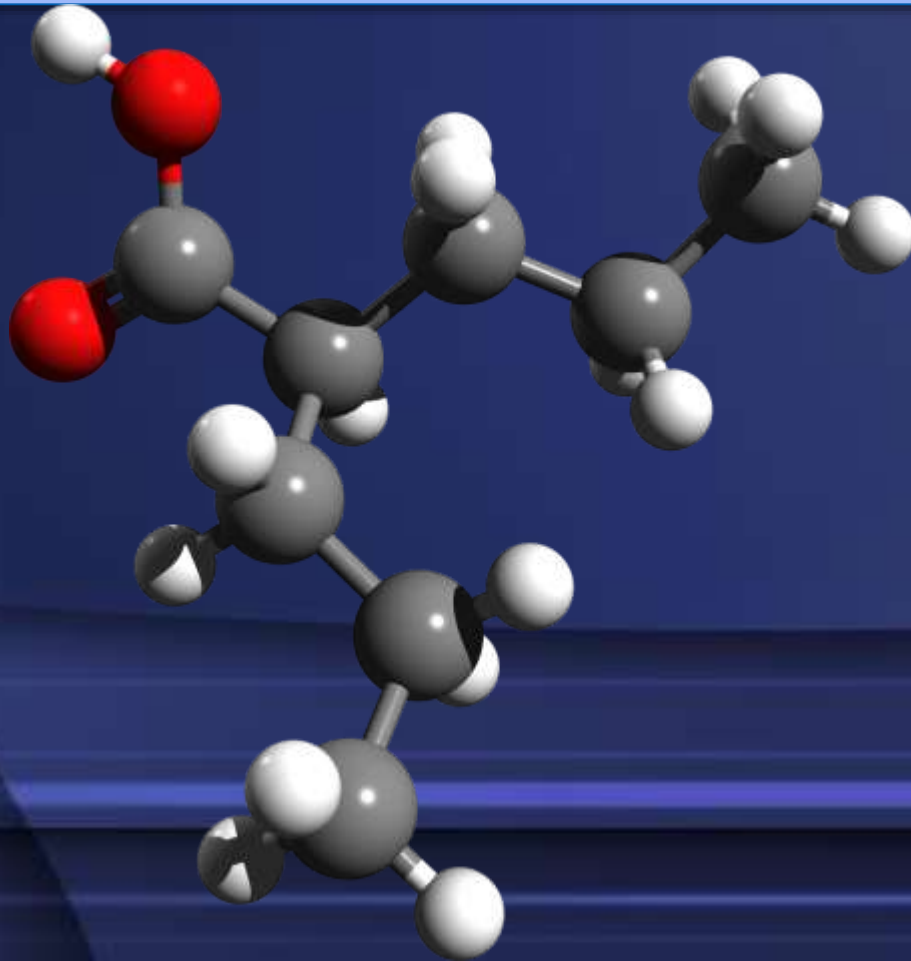
### **Demethylation of specific Wnt/ $\beta$ -catenin pathway genes and its upregulation in rat brain induced by prenatal valproate exposure.**

Wang Z<sup>1</sup>, Xu L, Zhu X, Cui W, Sun Y, Nishijo H, Peng Y, Li R.



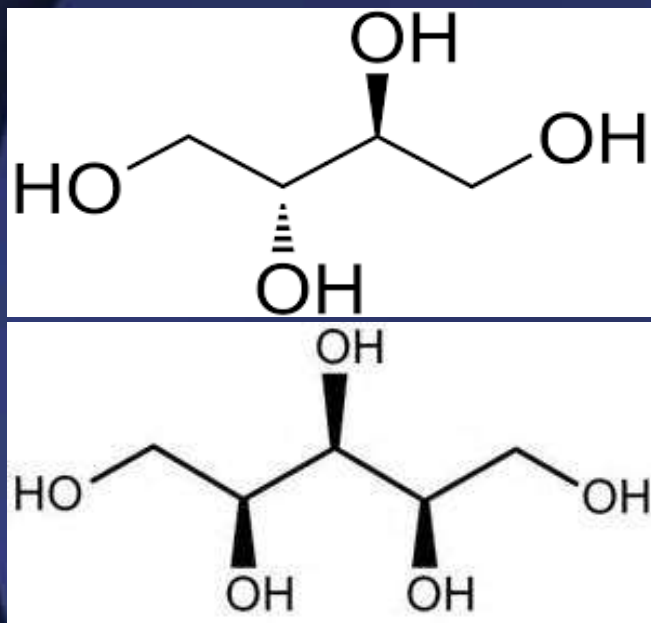
# Autism Spectrum Disorders

## Valproic and Propionic structures





# Ann and Robert Lurie Children's Hospital- Autism Spectrum Disorder Microbiome Research



- Do polyols have an inhibitory effect on the A.S.D. bacteria cultures?
- What **probiotic** inhibits the A.S.D. cultures?
- What promotes growth of the A.S.D. bacteria?



# Ann and Robert Lurie Children's Hospital- Autism Spectrum Disorder Microbiome Research

- Results: Eight strains were tested for polyol inhibitory activity C. histolyticum, B. vulgatis, C. bolteae (x2), C. difficile (x2), Bifidobacterium longham and Desulfovibrio. All strains grew to variable levels and had results that suggested polyol activity but did not reach a level of discernable growth to be able to assess the assays appropriately. Detailed OD values vs. polyol concentration are plotted as follows with relative inhibition inflection points.

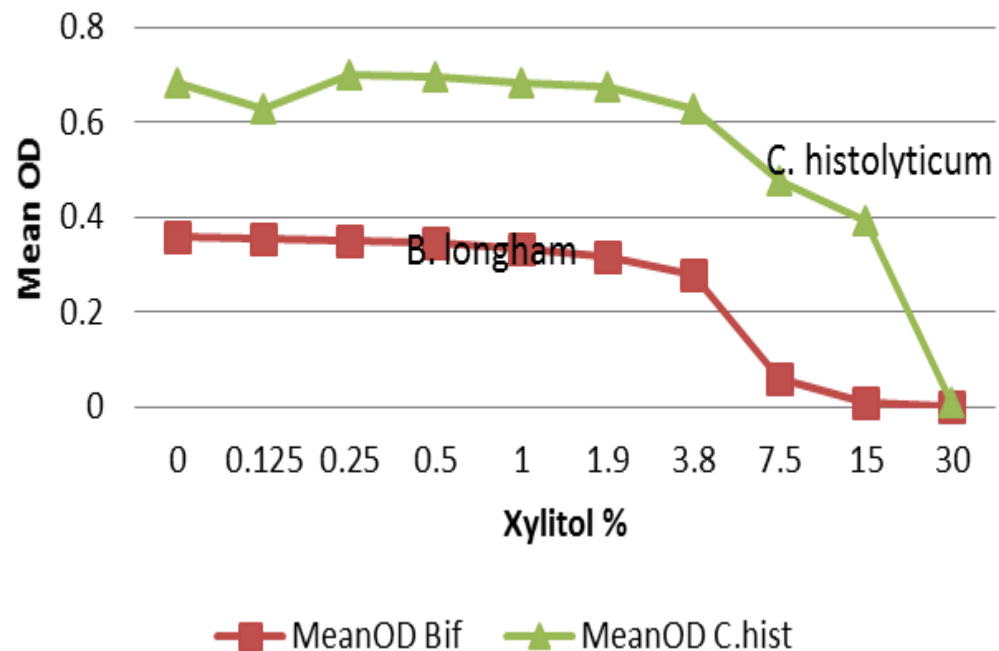
## Eight Strains- Two Polyols



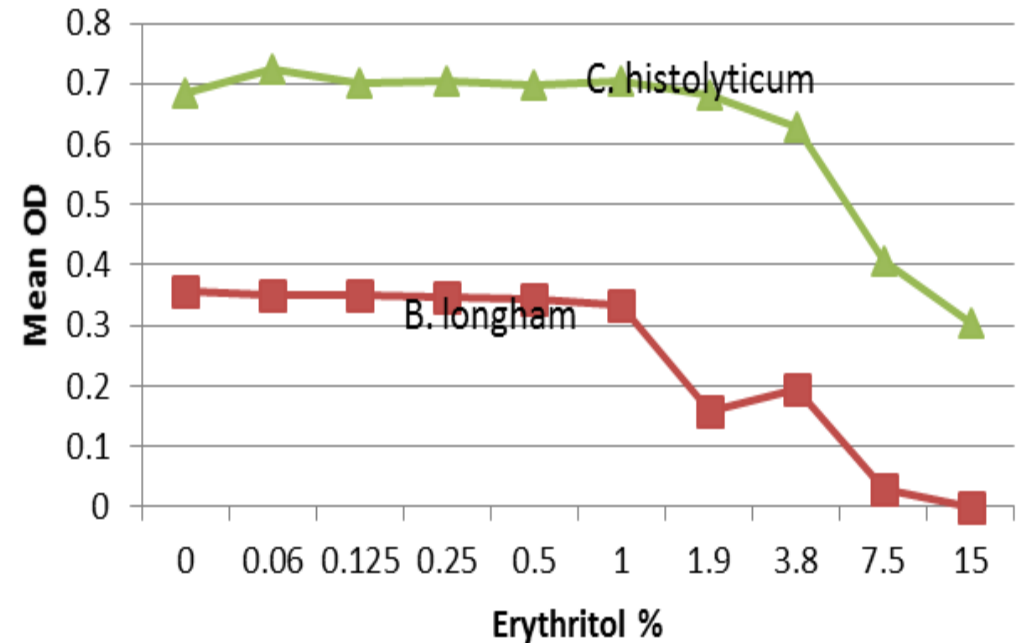
# Ann and Robert Lurie Children's Hospital- Autism Spectrum Disorder

## Microbiome Research

Xylitol inhibition of *B. longham* and *C. histolyticum*



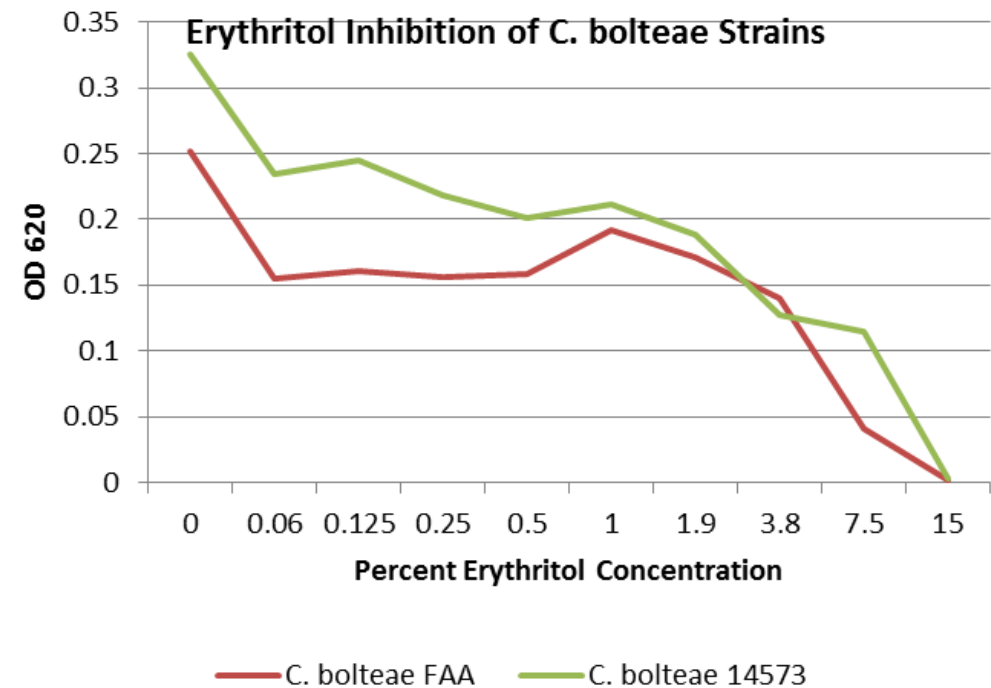
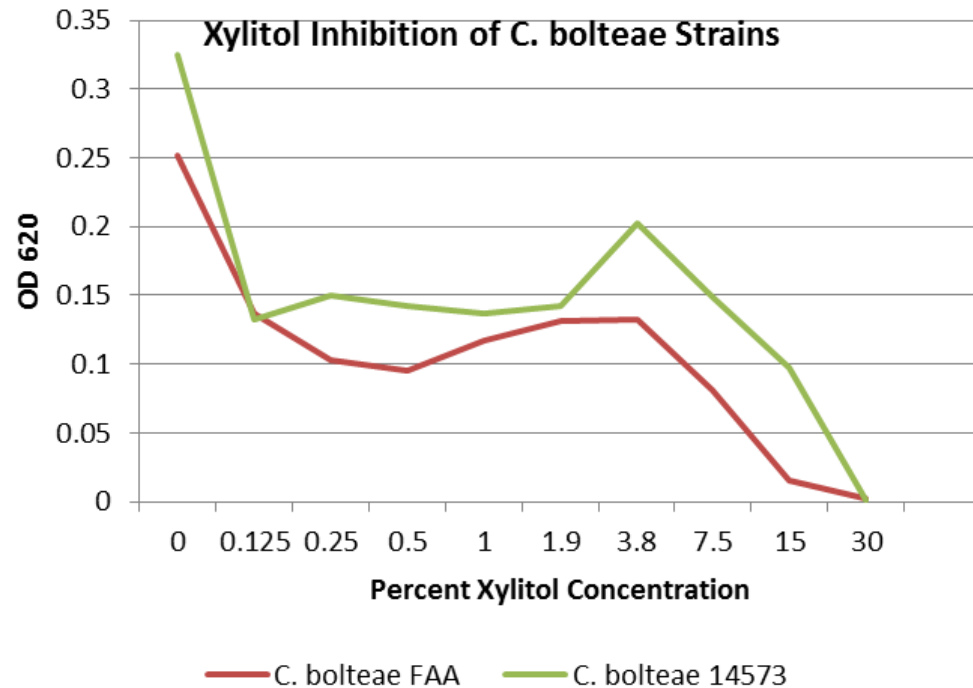
Erythritol Inhibition of *B. longham* and *C. histolyticum*



**Erythritol more effective**



# Ann and Robert Lurie Children's Hospital- Autism Spectrum Disorder Microbiome Research



- Erythritol seems better suited to inhibit *Clostridia bolteae*



# CDC puts C difficile burden at 453,000 cases, 29,000 deaths

Filed Under: [Antimicrobial Resistance](#); [Clostridium difficile](#)

Robert Roos | News Editor | CIDRAP News | Feb 25, 2015

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[🖨](#) Print & PDF

On the basis of a 2011 study, the Centers for Disease Control and Prevention (CDC) has increased its estimate of the annual burden of *Clostridium difficile* infections in the United States, putting it at 453,000 cases per year, with 29,300 associated deaths.

The agency, which released the findings in the *New England Journal of Medicine (NEJM)*, said they point up the need for better antibiotic stewardship and rigorous infection control in healthcare facilities.

*C diff* infections occur when someone is exposed to the pathogen while receiving antibiotic treatment for some other illness. Antibiotics suppress the normal bacteria in the colon, allowing *C diff* to flourish, producing toxins that cause severe diarrhea. Damage to the colon can cause bacteria to leak into the bloodstream.

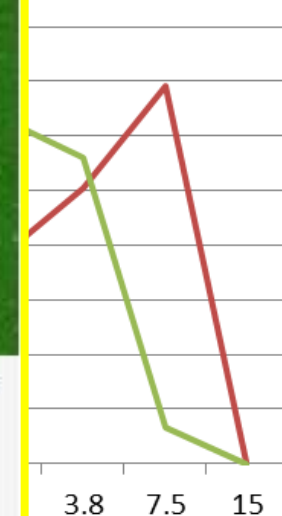
The CDC found that about two thirds of the 453,000 cases were related to a stay in a hospital or nursing home and the other third were community-associated cases, involving people with no recent hospital or nursing home exposure. Most of those who died were elderly.



CDC

Clostridium difficile bacteria, highly magnified.

Strains



ation

5557

- Xylit
- Perh

protocol?



# Nobel

The Gut-B  
programm  
behavior





# Microbiome and Epigenetics- so many ?s

## And so many publications...

J Am Acad Child Adolesc Psychiatry. 2010 Aug;49(8):794–809. doi: 10.1016/j.jaac.2010.05.005. Epub 2010 Jul 3.

### Autism spectrum disorders and epigenetics.




Grafodatskaya D<sup>1</sup>, Chung B, Szatmari P, Weksberg R.

epigenetics one of multifactorial etiologies of ASD

#### REVIEW ARTICLE

Front. Neurol., 26 May 2015 | <http://dx.doi.org/10.3389/fneur.2015.00107>

## The role of epigenetic change in autism spectrum disorders

 Yuk Jing Loke<sup>1</sup>,  Anthony John Hannan<sup>2</sup> and  Jeffrey Mark Craig<sup>1\*</sup>

<sup>1</sup>Murdoch Childrens Research Institute, Royal Children's Hospital and Department of Paediatrics, University of Melbourne, Parkville, VIC, Australia

<sup>2</sup>Melbourne Brain Centre, Florey Institute of Neuroscience and Mental Health, The University of Melbourne, Parkville, VIC, Australia

ASD, thereby supporting a role for epigenetics in the multifactorial etiologies of ASD.

# Epigenetics

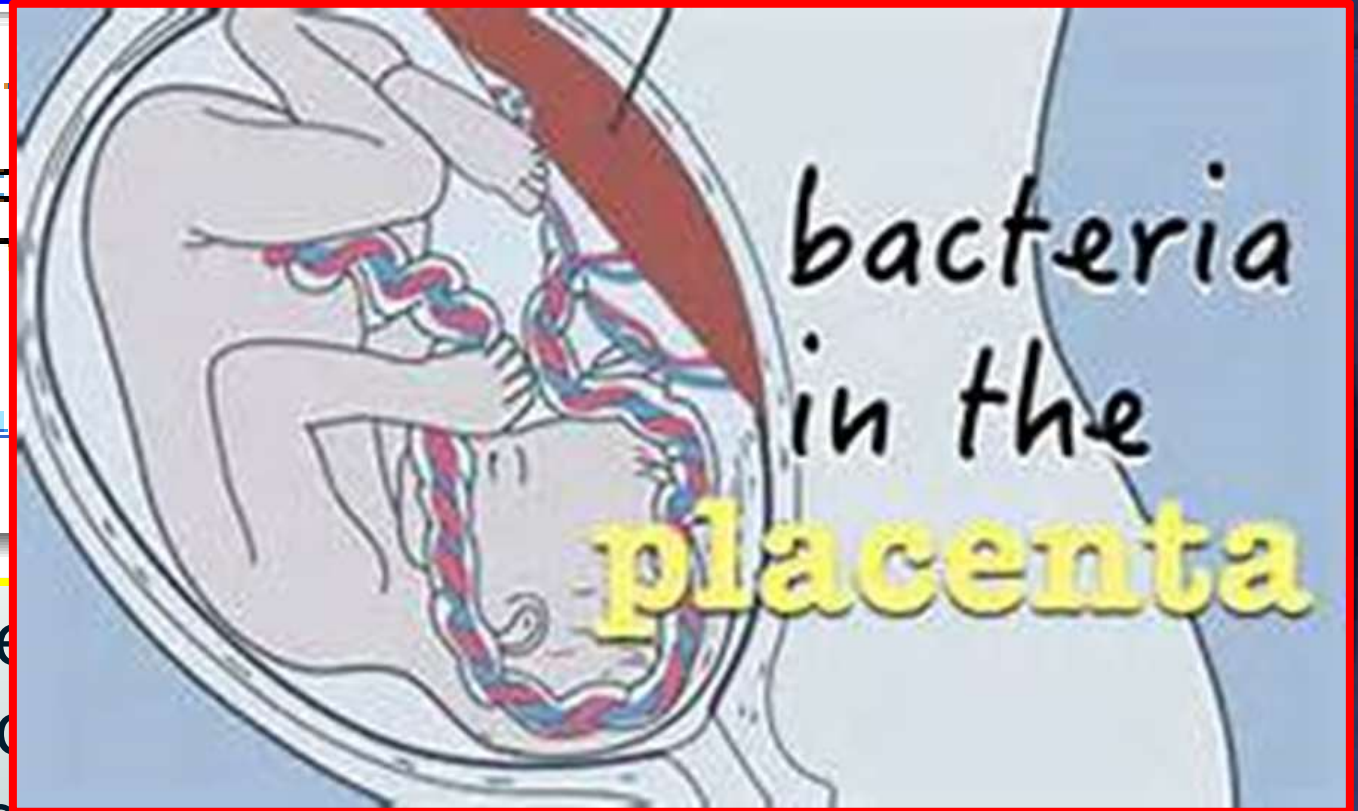


# DNA methylation and Autism

*Molecular Psychiatry* (2014) **19**, 495–503; doi:10.1038/mp.2014.10

## Methylomic analysis of monozygotic twins discordant for autism spectrum disorder and controls

C C Y Wong<sup>1</sup>, E L Meaburn<sup>1,2</sup>, A Ronald<sup>1</sup>,  
C Schalkwyk<sup>1</sup>, R Plomin<sup>1</sup> and J Mill<sup>1,4</sup>



Genome-wide analysis of DNA methylation in monozygotic twin pairs (100 individuals) sampled from the Environmental Risk Longitudinal Twin Study that included twins discordant and concordant for ASD, ASD-associated traits and no autistic phenotype.

Significant correlations between DNA methylation and quantitatively measured autistic trait scores across our sample cohort. This study represents the first systematic epigenomic analyses of MZ twins discordant for ASD and implicates a role for altered DNA methylation in autism.





# Modulation of Immunological Pathways in Autistic and Neurotypical Lymphoblastoid Cell Lines by the Enteric Microbiome Metabolite Propionic Acid

Richard E. Frye<sup>1,2\*</sup>, Bistra Nankova<sup>3</sup>, Sudeepa Bhattacharyya<sup>1,2</sup>, Shannon Rose<sup>1,2</sup>, Sirish C. Bennuri<sup>1,2</sup> and Derrick F. MacFabe<sup>4</sup>

**Propionic acid (PPA)** is a ubiquitous short-chain fatty acid which is a fermentation product of the enteric microbiome and present or added to many foods. While PPA has beneficial effects, it is also associated with human disorders, including autism spectrum disorders (ASDs). We previously demonstrated that PPA modulates mitochondrial dysfunction differentially in subsets of lymphoblastoid cell lines (LCLs) derived from patients with ASD. Specifically, PPA significantly increases mitochondrial function in LCLs that have mitochondrial dysfunction at baseline [individuals with autistic disorder with atypical mitochondrial function (AD-A) LCLs] as compared to ASD LCLs with normal mitochondrial function [individuals with autistic disorder with normal mitochondrial function (AD-N) LCLs] and control (CNT) LCLs.



ARTICLE

Open Access

# Butyrate enhances mitochondrial function during oxidative stress in cell lines from boys with autism

Shannon Rose<sup>1</sup>, Sirish C. Bennuri<sup>1</sup>, Jakeira E. Davis<sup>1</sup>, Rebecca Wynne<sup>1</sup>, John C. Slattery<sup>1</sup>, Marie Tippet<sup>1</sup>, Leanna Delhey<sup>1</sup>, Stephan Melnyk<sup>1</sup>, Stephen G. Kahler<sup>1</sup>, Derrick F. MacFabe<sup>2</sup> and Richard E. Frye<sup>1,3</sup>

In general, these data suggest that **BT can enhance mitochondrial function** in the context of physiological stress and/or mitochondrial dysfunction, and may be an important metabolite that can help **rescue energy metabolism during disease states**. Thus, insight into this metabolic modulator may have wide applications for both health and disease since **BT has been implicated in a wide variety of conditions including ASD**.

However, future clinical studies in humans are needed to help define the practical implications of these physiological findings.





# Restoring Mitochondrial Function- polyols? Then probiotics?

Biomark Med. 2015 Oct;9(10):957-65. doi: 10.2217/bmm.15.72. Epub 2015 Oct 6.

## Mitochondrial enzyme dysfunction in autism spectrum disorders; a novel biomarker swab analysis.

Goldenthal MJ<sup>1,2</sup>, Damle S<sup>1</sup>, Sheth S<sup>1</sup>, Shah N<sup>1</sup>, Melvin J<sup>2</sup>, Jethva R<sup>2</sup>, Hardison H<sup>2</sup>, Marks H<sup>3</sup>, Leoido A<sup>2</sup>.

### ⊕ Author information

#### Abstract

**AIM:** Mitochondrial function studies in autism spectrum disorders (ASD) have detected skeletal muscle mitochondrial respiratory complex (RC) activities. As a muscle biopsy is expensive and invasive, we assessed RC-I and

**METHODS:** 92 children with ASD and 68 controls were studied with immunocapture for RC-I and microspe

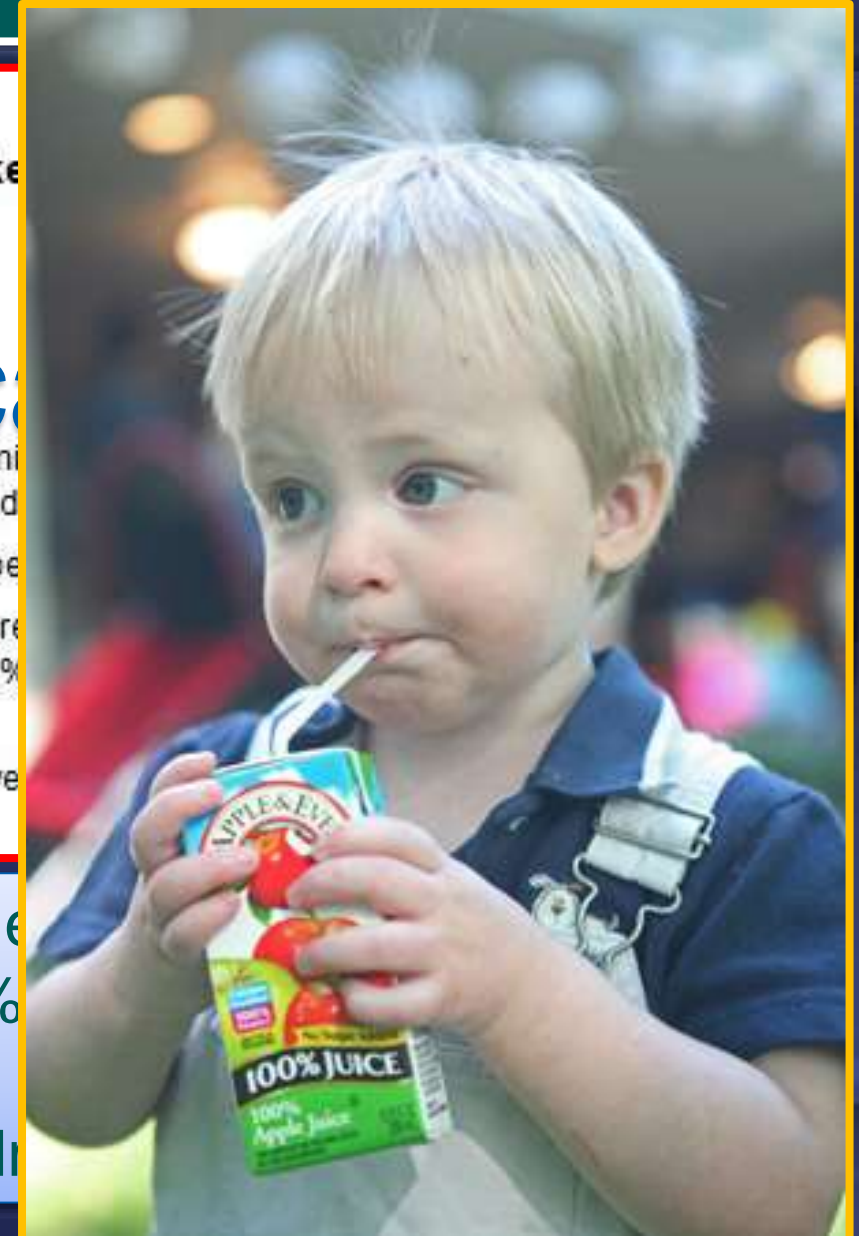
**RESULTS:** Significant RC activity deficiencies were found in 39 (42%) ASD patients ( $p < 0.01$ ) and more pre  
Aberrant RC overactivity was seen in 9 children. RC-I/RC-IV activity ratio was significantly increased in 64%  
76% of those more severely affected ( $p < 0.05$ ).

**CONCLUSION:** Buccal swab analysis revealed extensive RC abnormalities in ASD providing a noninvasive  
function in ASD patients.

# Age One visit- Buccal

42% have significant RC deficiencies  
RC-I/RC-IV activity ratio significantly increased in 64%  
severe ASD

MUST BE CONTRIBUTORY!!!! Restore Mitochondr





# Simpson Querrey Center for Epigenetics



Louis A. Simpson and Kimberly K. Querrey Biomedical Research Center, a 14-story, 600,000-square-foot building that will significantly expand Feinberg's biomedical research enterprise.

- “We study the effects of environment on the activation and expression of genes.”



# SCFA- microbiome

- **Pilot study of the SCFA  
Headspace Analysis of Bacterial  
Metabolites in Media with and  
without Polyols**
- **MacFabe, D., Habibi, Kabat, B.,  
Cannon, M., Gashkoff, M., Zurek, R.**





# SCFA- microbiome

- Brain Heart Infusion Broth (BHI2 or BHI10) supplemented with 2% or 10% sucrose containing no polyols or either erythritol or xylitol at various concentrations was used for this study. Streptococcus mutans (ATCC 35668) was grown aerobically. After 48 hours of growth the supernatant were harvested and centrifuged to pellet bacteria. Supernatants were removed from bacterial pellets, filtered through 0.22 micron filters and stored in sterile cryovials until submitted for Short Chain Fatty Acid (SCFA) analysis at the IMSERC Mass Spectrometry Center (Northwestern University).

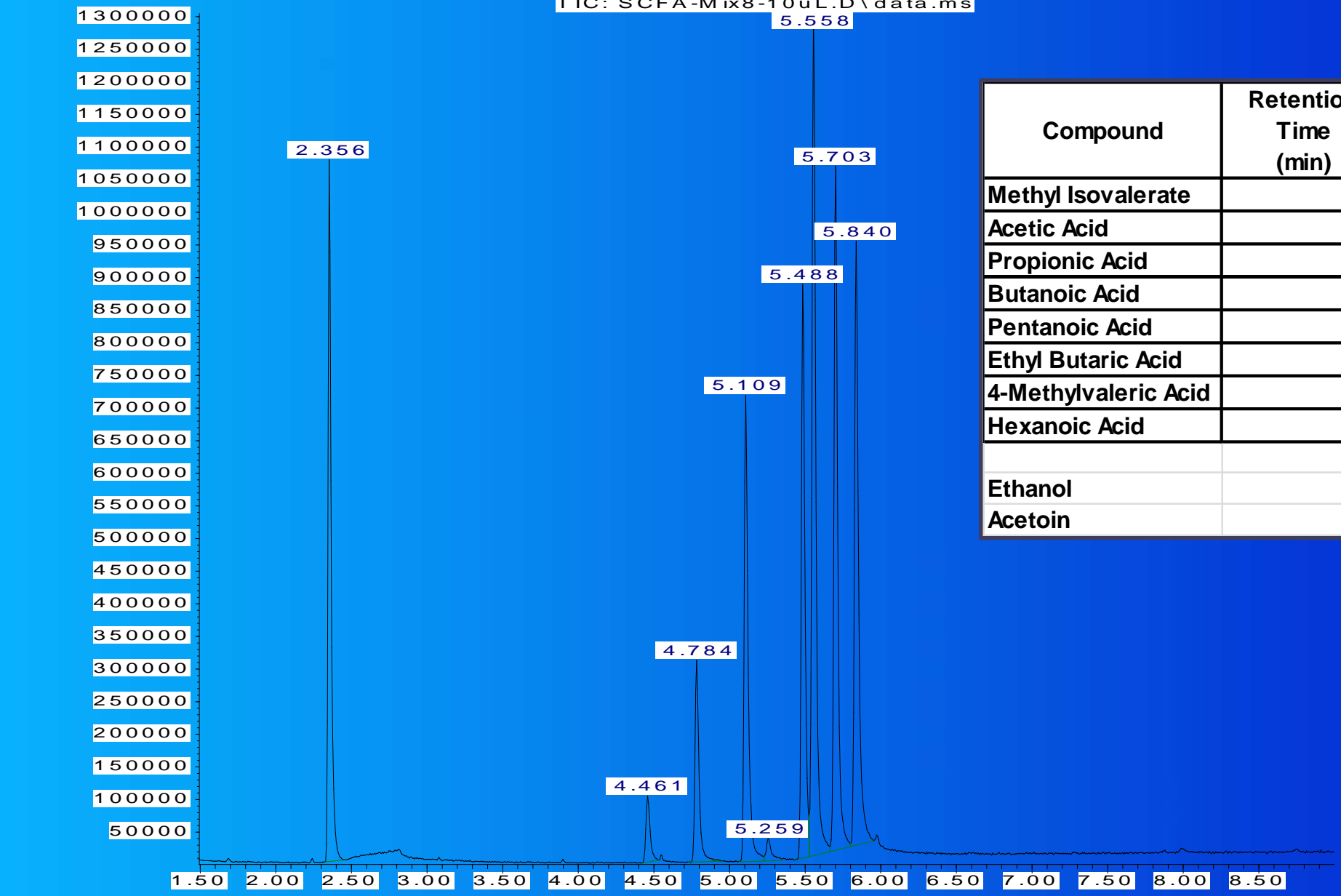




Standard Mix: 52 ng on column

Abundance

TIC: SCFA-Mix8-10uL.D\data.ms



Time-->

Compound	Retention Time (min)	m/z
Methyl Isovalerate	2.4	74
Acetic Acid	4.5	60
Propionic Acid	4.8	74
Butanoic Acid	5.1	60
Pentanoic Acid	5.5	60
Ethyl Butaric Acid	5.6	88
4-Methylvaleric Acid	5.7	57
Hexanoic Acid	5.9	60
Ethanol	2.0	45
Acetoin	3.8	45

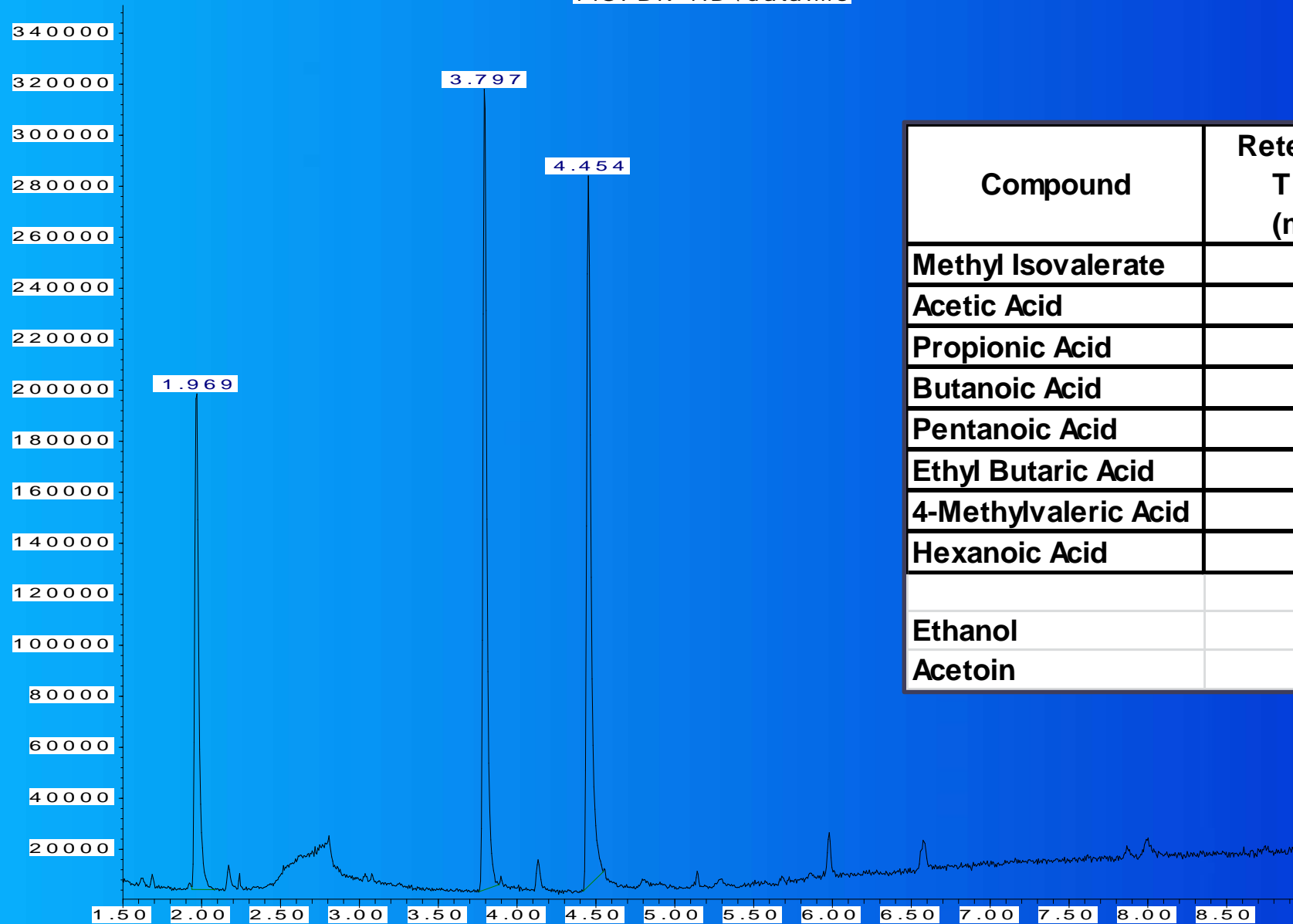




Abundance

Sample BK-4: Erythritol-1.25mg

TIC: BK-4.D\data.ms



Time-->

Compound	Retention Time (min)	m/z
Methyl Isovalerate	2.4	74
Acetic Acid	4.5	60
Propionic Acid	4.8	74
Butanoic Acid	5.1	60
Pentanoic Acid	5.5	60
Ethyl Butaric Acid	5.6	88
4-Methylvaleric Acid	5.7	57
Hexanoic Acid	5.9	60
Ethanol	2.0	45
Acetoin	3.8	45





# SCFA- microbiome

- Constituents of media effect the bacterial metabolite production, possibly shifting from benign or protective to more pathogenic. Additional laboratory study is required testing other species, specifically the **propionic producing *Clostridium histolyticum* and *boltae* plus *Bacteroides vulgatus***.





## Alterations of oral microbiota distinguish children with autism spectrum disorders from healthy controls

Yanan Qiao, Mingtao Wu, Yanhuizhi Feng, Zhichong Zhou, Lei Chen & Fengshan Chen 

*Scientific Reports* **8**, Article number: 1597

Received: 23 February 2017



- Moreover, pathogens such as Haemophilus in saliva and Streptococcus in plaques showed significantly higher abundance in ASD patients, whereas commensals such as Prevotella, Selenomonas, Actinomyces, Porphyromonas, and Fusobacterium were reduced. Specifically, an overt depletion of Prevotellaceae co-occurrence network in ASD patients was obtained in dental plaques. The distinguishable bacteria were also correlated with clinical indices, reflecting disease severity and the oral health status (i.e. dental caries). Finally, diagnostic models based on key microbes were constructed, with 96.3% accuracy in saliva.



# Oral Microbiome- Autism



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ScienceDirect

Journal homepage: [www.intl.elsevierhealth.com/journals/jden](http://www.intl.elsevierhealth.com/journals/jden)



CrossMark

## Effect of three-year consumption of erythritol, xylitol and sorbitol candies on various plaque and salivary caries-related variables

Riina Runnel<sup>a,\*</sup>, Kauko K. Mäkinen<sup>b</sup>, Sisko Honkala<sup>c</sup>, Jana Olak<sup>a</sup>,  
Pirkko-Liisa Mäkinen<sup>b</sup>, Rita Nömmela<sup>a</sup>, Tero Vahlberg<sup>d</sup>,  
Eino Honkala<sup>b,c</sup>, Mare Saag<sup>a</sup>

- Three-year consumption of erythritol-containing candies by initially 7- to 8-year old children was associated with reduced plaque growth, lower levels of plaque, acetic acid and propionic acid, and reduced oral counts of mutans streptococci compared with the consumption of xylitol or sorbitol candies.





# Microbial GPS- maternal and buccal swabs

MITOCHONDRIAL DYSFUNCTION MAY  
BE LINKED TO NEUROLOGICAL DISORDERS

- Autism
- ADHD
- Alzheimer's
- Parkinson's Disease
- ME/CFS
- Migraine Headaches
- Seizures

Early Diagnosis can  
lead to Effective  
Treatment and  
Better Outcomes



/2017

Center

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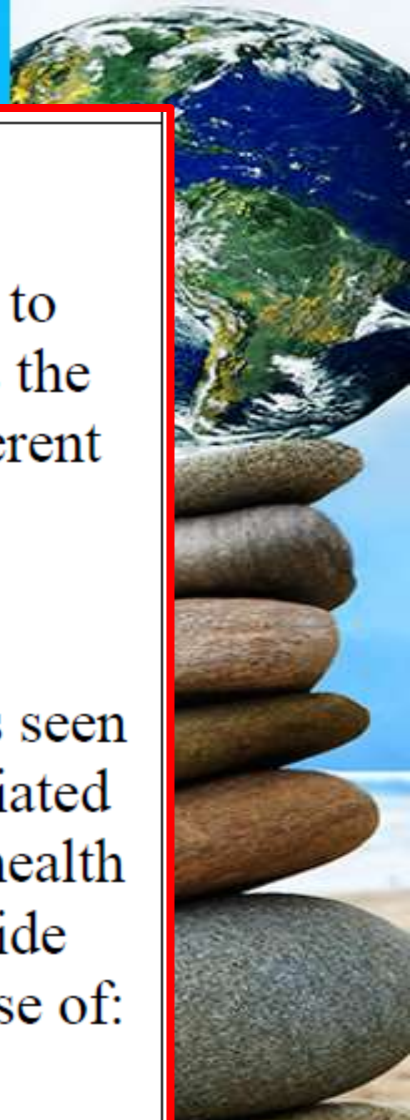
# We are short funds....

## Objectives

Part 1- Mitochondrial health, especially in children with A.S.D., needs to be evaluated, before and after supplementation. Microbiome changes that may occur due to supplementation need to be determined accurately, as does the existing oral microbiome differences that occur in the different study groups prior to any supplementation.

Part 2- The population of the United States of America has seen a dramatic increase in the incidence of many immune mediated diseases causing a near crisis burden upon its society and health care system. The two distinct Cuban populations will provide for the complete investigation and the comparison of the use of: food preservatives, western agriculture, mass produced prepared food, and the overuse of antibiotics on the microbiome, both nasal and oral.

and funds are going elsewhere!





# Nobel Conference 2017



Mo

r





# Take Home- probiotics



- Special needs patients may all have different microbiomes- A.S.D. patients definitely have a shifted gut and oral microflora and most likely benefit from polyol therapy





# Probiotics and Microbiome

- Allergies



1 in 13 children  
in the U.S. has  
a food allergy...



That's roughly  
**6 million**  
children.





# Allergies.... BCBS data

ALLERGIES  
now affect

18%

of children in the U.S.

**Ridiculous from Evolutionary  
Viewpoint**





# Allergies and Ectopic Dermatitis- Barrier Microbiome

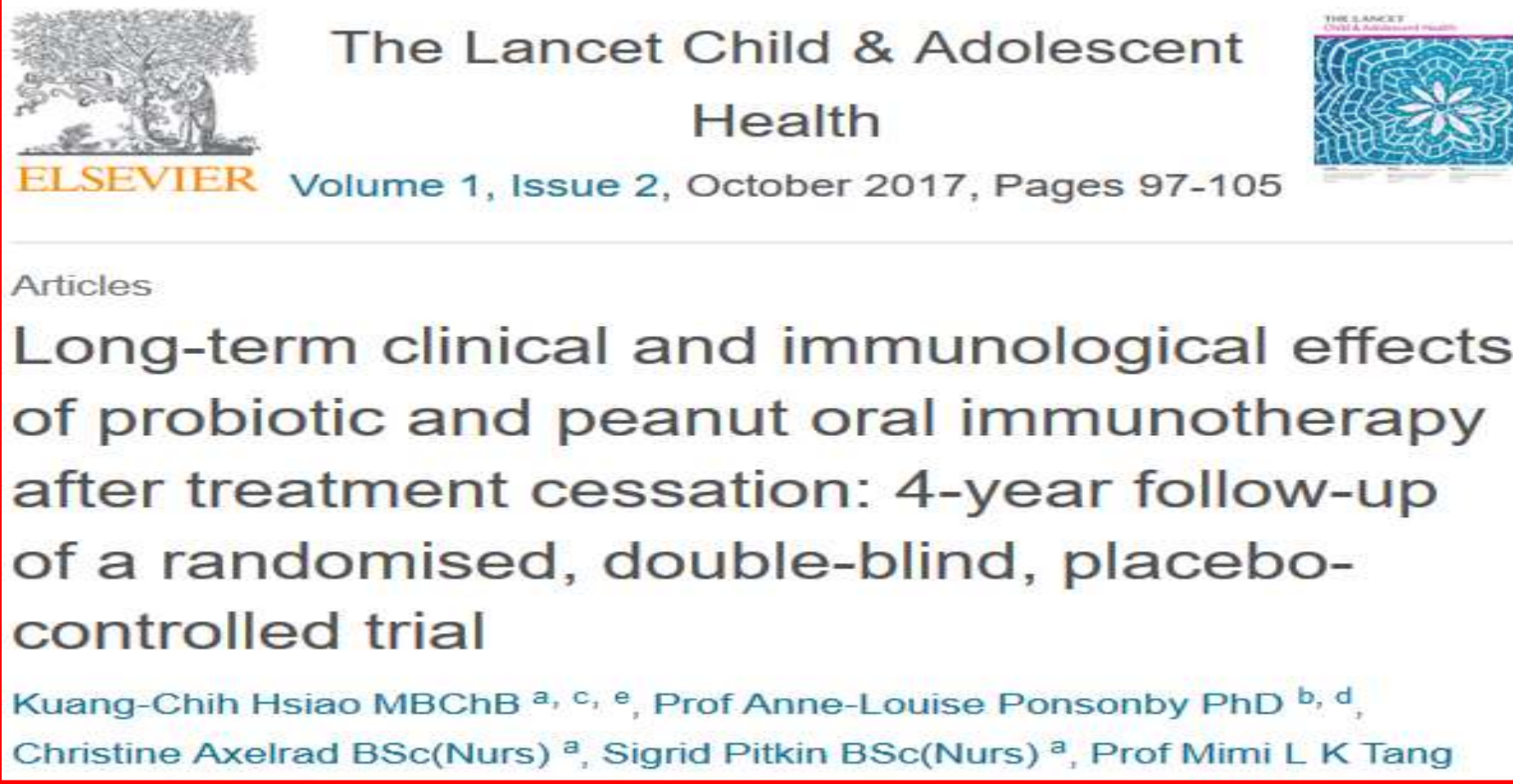


Dysbiosis  
creates food  
allergies!

The **alarming increase in the incidence and severity of food allergies has coincided with lifestyle changes** in Western societies, such as dietary modifications and increased antibiotic use. There is increasing evidence that the **dysbiosis associated with sensitization to food fails to stimulate protective tolerogenic pathways**, leading to the development of the type 2 immune responses that characterize allergic disease.



Res  
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Peanut Allergy  
in children



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To conclude, our results suggest that PPOIT is effective at inducing long-term sustained unresponsiveness that persists for up to **4 years after completing treatment and is safe**. Furthermore, the finding that sustained unresponsiveness was maintained without the need to follow a regular prespecified ingestion schedule provides a compelling argument that PPOIT-induced immune tolerance.

Natural rate of desensitisation for peanut allergy.



# Take Home- probiotics



- Food allergies can be due to Dysbiosis and removal of protective barriers
- Probiotics have been proven to help with food allergies.







# Dietary fiber?????



World Journal of  
Gastroenterology

World J Gastroenterol. 2012 Sep 7; 18(33): 4593–4596.

Published online 2012 Sep 7. doi: [10.3748/wjg.v18.i33.4593](https://doi.org/10.3748/wjg.v18.i33.4593)

PM

## Stopping or reducing dietary fiber intake reduces constipation and its associated symptoms

Kok-Sun Ho, Charmaine You Mei Tan, Muhd Ashik Mohd Daud, and Francis Seow-Choen



- Patients who stopped or reduced dietary fiber had significant improvement in their symptoms** while those who continued on a high fiber diet had no change. Of those who stopped fiber completely, the bowel frequency increased from one motion in 3.75 d ( $\pm 1.59$  d) to one motion in 1.0 d ( $\pm 0.0$  d) ( $P < 0.001$ ); those with reduced fiber intake had increased bowel frequency from a mean of one motion per 4.19 d ( $\pm 2.09$  d) to one motion per 1.9 d ( $\pm 1.21$  d) on a reduced fiber diet ( $P < 0.001$ ); those who remained on a high fiber diet continued to have a mean of one motion per 6.83 d ( $\pm 1.03$  d) before and after consultation. **For no fiber, reduced fiber and high fiber groups, respectively, symptoms of bloating were present in 0%, 31.3% and 100% ( $P < 0.001$ ) and straining to pass stools occurred in 0%, 43.8% and 100% ( $P < 0.001$ ).**



# Probiotics and Microbiome

## • Obesity





# Obesity- pro

SCIENTIFIC R

Article | OPEN | Published: 19 September

## Child Weight Gain Oral Microbiota C

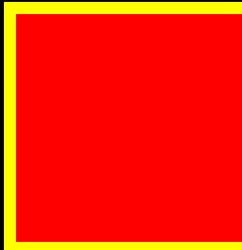
Sarah J. C. Craig, Daniel Blankenberg, Alic  
Savage, Michele E. Marini, Jennifer L. Stok  
Chiaromonte ✉ & Kateryna D. Makova ✉

Scientific Reports 8, Article number: 14030



Lastly, we identified several bacterial genera that were associated with child growth patterns. These results suggest that by the age of two, the oral microbiota of children with rapid infant weight gain may have already begun to establish patterns often seen in obese adults.





CNN Money

Companies

## McDonald's from its buns

by Jordan Valinsky @CNMoney

🕒 September 27, 2018: 10:07 AM ET

- McDonald's (M) calcium propionate from its buns and from its American cheese.

The buns will no longer have the artificial preservative calcium propionate. In general, calcium propionate helps prevent mold growth on bread and is considered antifungal. McDonald's may be getting rid of it because previous research found that this preservative could negatively affect children's behavior. The study, published in the Journal of Pediatrics and Child Health, showed that calcium propionate might cause "irritability, restlessness, inattention and sleep disturbance in some children." In addition, removing the preservative could reverse these behavioral problems.- Forbes Magazine





QUARTER POUNDER  
MUFFIN  
within 7 days  
or visit.

receipt date:  
McDonald's.

00112-3

2648

3

12:43 PM  
order 57

5.29

1.00

3.99

10.28

0.95

1.23

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23



# Weight/diabetes- probiotics

ARTICLE | VOLUME 6, ISSUE 2, P157-170.E8, FEBRUARY 28, 2018

## Integrative Personal Omics Profiles during Periods of Weight Gain and Loss

Brian D. Piening <sup>16</sup> • Wenyu Zhou <sup>16</sup> • Kévin Contrepois <sup>16</sup> • ... Tracey L. McLaughlin <sup>16</sup> • George M. Weinstock <sup>16</sup> • Michael P. Snyder <sup>16, 17</sup> • Show all authors • Show footnotes

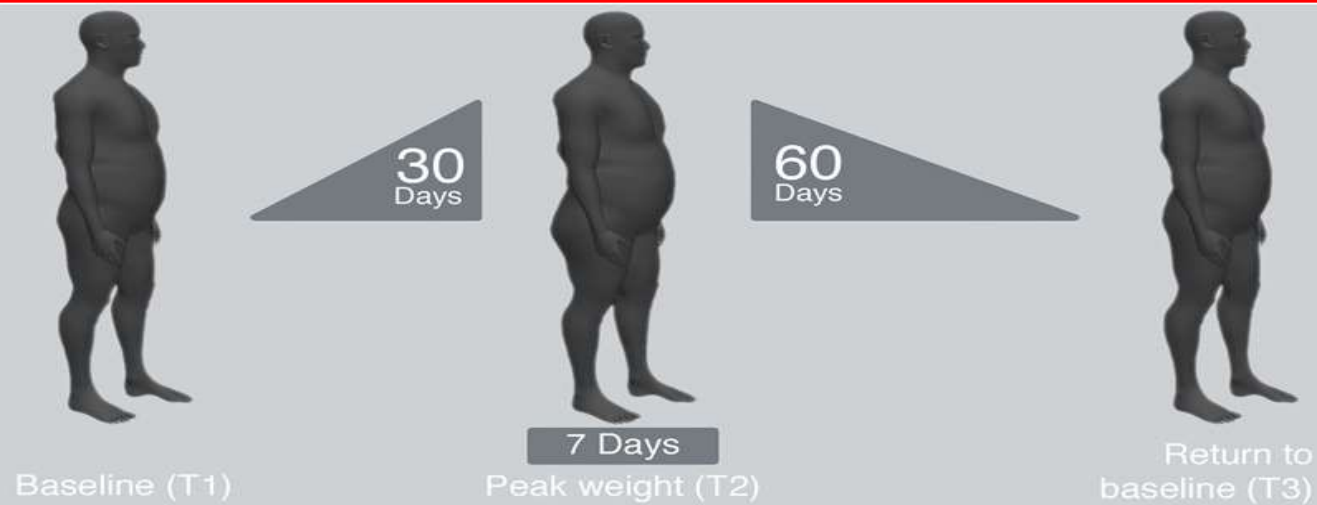
Published: January 23, 2018 • DOI: <https://doi.org/10.1016/j.cels.2017.12.013> •

Cell Systems

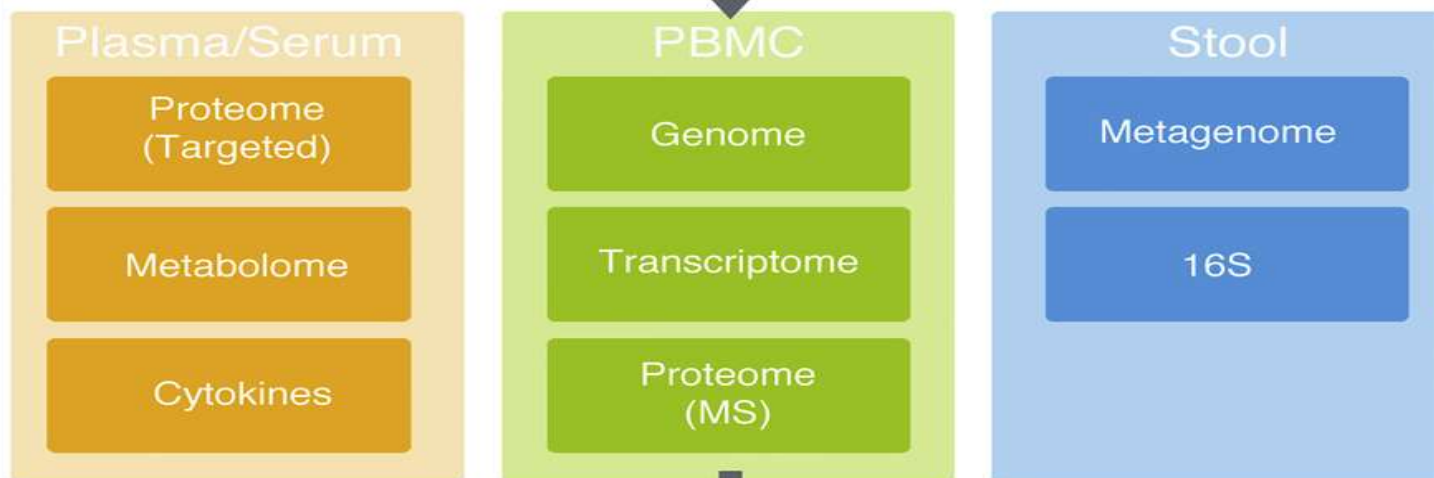
- Performed a controlled longitudinal weight perturbation study combining multiple omics strategies (genomics, transcriptomics, multiple proteomics assays, metabolomics, and microbiomics) during periods of weight gain and loss in humans.



Weight perturbation



Omics analyses



Data integration

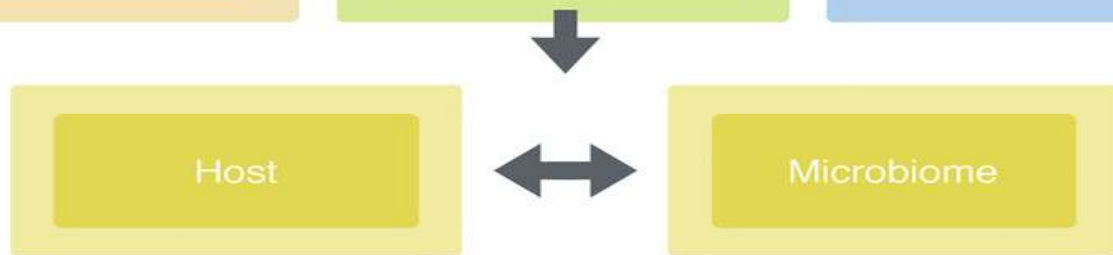
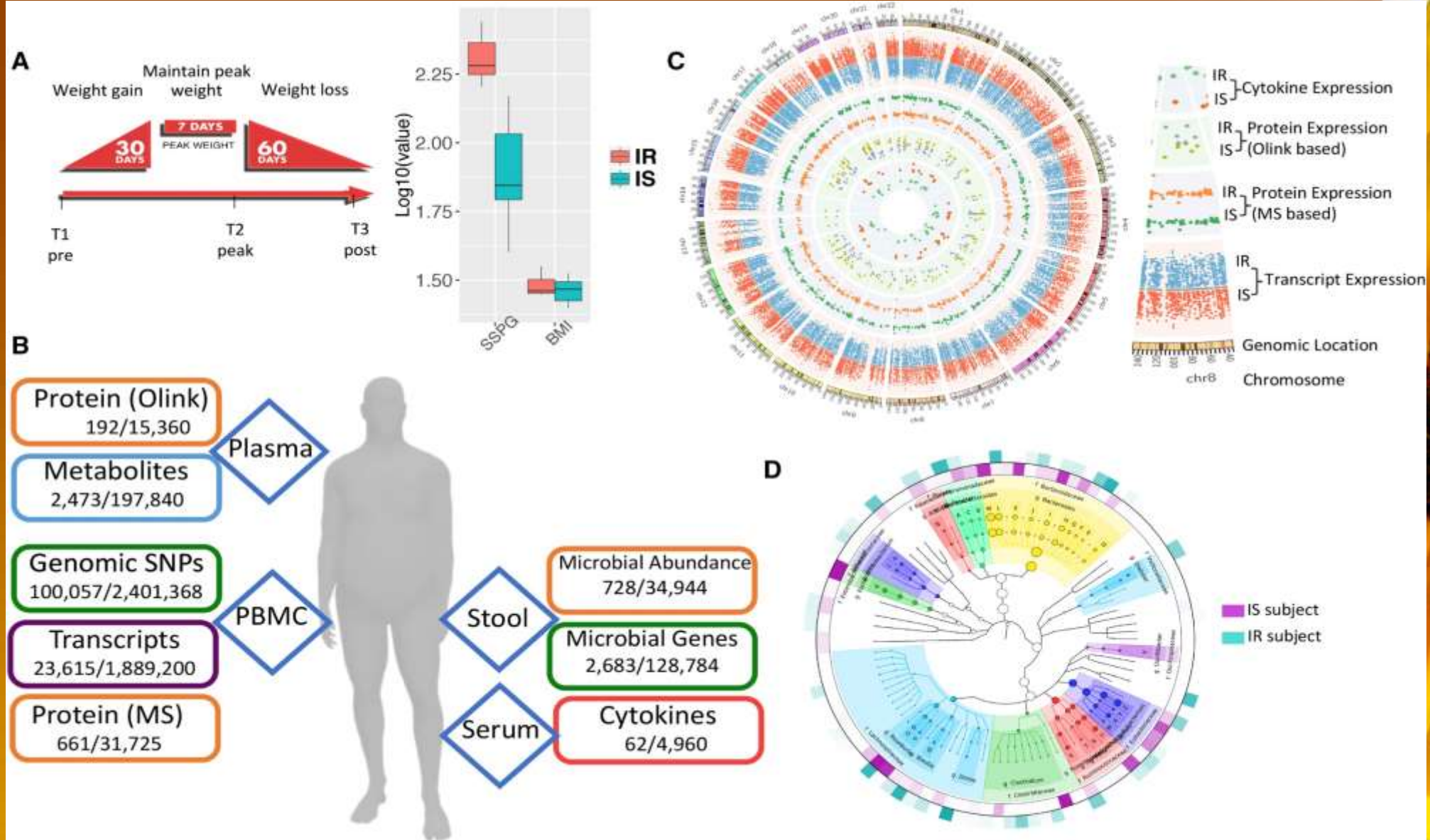




Figure 1





# Integrative Personal Omics Profiles during Periods of Weight Gain and Loss

Cell Systems

Brian D. Piening <sup>16</sup> • Wenyu Zhou <sup>16</sup> • K vin Contrepois <sup>16</sup> • ... Tracey L. McLaughlin <sup>16</sup>  
George M. Weinstock <sup>16</sup> • Michael P. Snyder <sup>16, 17</sup> • [Show all authors](#) • [Show footnotes](#)

Published: January 23, 2018 • DOI: <https://doi.org/10.1016/j.cels.2017.12.013> • [Check for updates](#)

- We also found extensive molecular changes after weight gain and weight loss. Notably the inflammation response was one of the major pathways induced upon weight gain; This dysregulation is evident at several different levels, including transcriptome, proteome, and cytokines. These results suggest that a **systemic inflammatory pathway is activated in response to short-term weight gain**, which is surprising given the modest weight gain induced here. it is interesting to note that increases in gram-positive **Firmicutes correlate with increased inflammation** in this study, raising the possibility of other non-LPS triggers of a low- level systemic immune response in overweight/obese humans.



# Integrative Personal Omics Profiles during Periods of Weight Gain and Loss

Cell Systems

Brian D. Piening <sup>16</sup> • Wenyu Zhou <sup>16</sup> • Kévin Contrepois <sup>16</sup> • ... Tracey L. McLaughlin <sup>16</sup> ✉ • George M. Weinstock <sup>16</sup> ✉ • Michael P. Snyder <sup>16, 17</sup> ✉ • [Show all authors](#) • [Show footnotes](#)

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Using both 16S and shotgun metagenomics of the stool microbiome, we observed significant differences between IR and IS participants in the abundance of the gram-negative proteobacterium ***Oxalobacter formigenes*** ( $p < 0.006$ ). Interestingly, although this bacterium was present at relatively high levels in IS participants, it was not detected in any of the IR participants' samples. *O. formigenes* is particularly unusual in that it processes oxalate, and absence of this bacterium is associated with increased risk of kidney stones which was linked to diabetes and insulin resistance and can be sensitive to high-oxalate-containing foods such as almonds.



# Renal- probiotics



Mary Ann Liebert, Inc. publishers

JOURNAL OF ENDOUROLOGY

Journals

Search

Alerts

J Endourol. 2011 Apr; 25(4): 673–679.

doi: [10.1089/end.2010.0462](https://doi.org/10.1089/end.2010.0462)

PMCID: PMC3071521

PMID: [21381959](https://pubmed.ncbi.nlm.nih.gov/21381959/)

## Factors Related to Colonization with *Oxalobacter formigenes* in U.S. Adults

Judith Parsells Kelly, M.S.,<sup>1</sup> Gary C. Curhan, M.D., Sc.D.,<sup>2</sup> David R. Cave, M.D., Ph.D.,<sup>3</sup> Theresa E. Anderson, R.N.,<sup>1</sup>

The overall prevalence of *O. formigenes* was 38%. Use of **specific antibiotics previously thought to affect the bacterium was significantly related to colonization, with prevalences of 17%, 27%, and 36%, for those who had used these drugs <1, 1–5, and >5 years ago,** compared with 55% in nonusers. There were no significant associations with demographic factors, nutrient intake, or medical history, although the prevalence appeared to increase somewhat with increasing oxalate consumption.



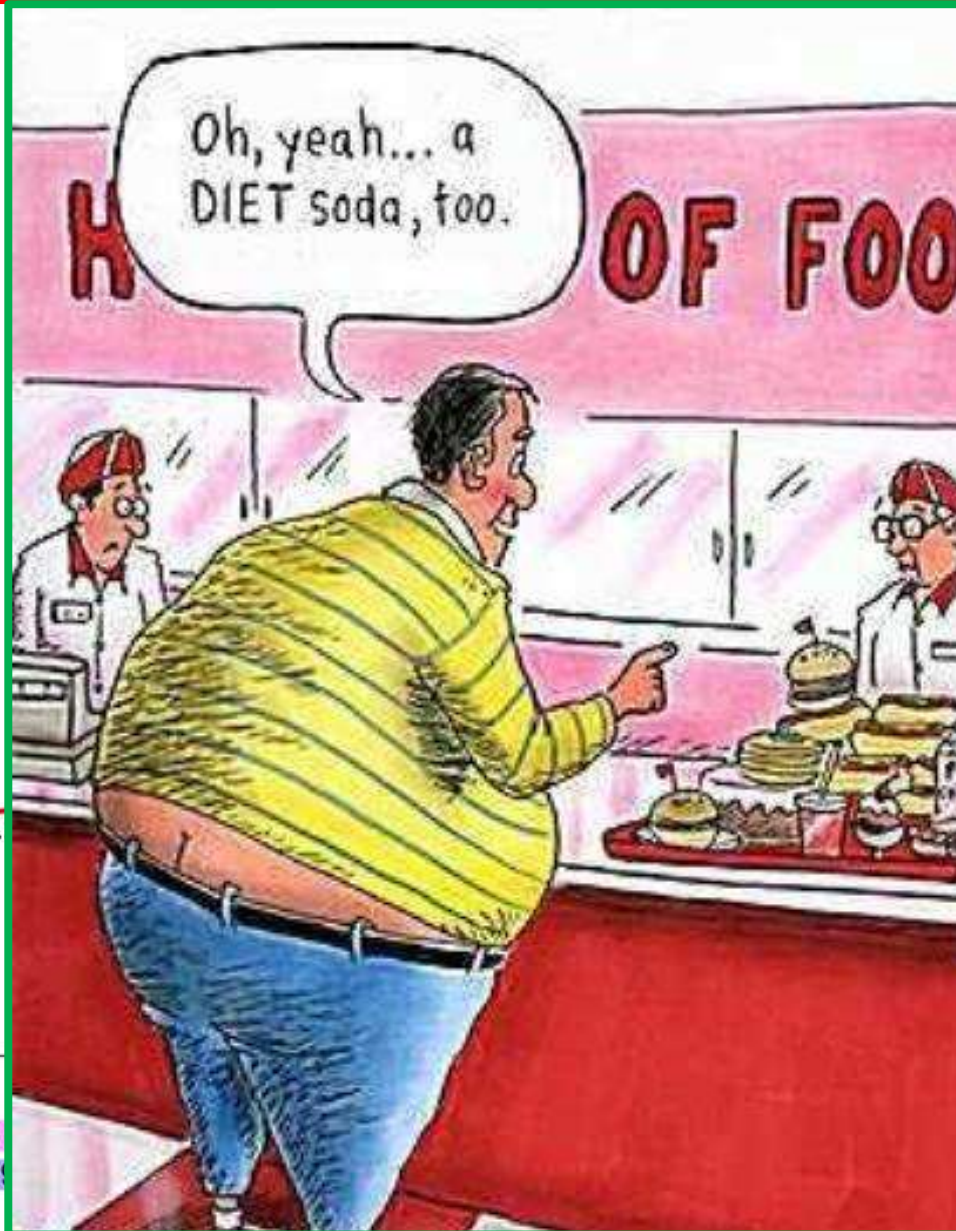
# Probiotics and Microbiome

- **NAS**
  - **Non caloric**  
**Artificial**  
**Sweetener**





# Probiotics and Diet



YALE JOURNAL OF  
BIOLOGY AND MEDICINE

cial sweeteners and the neuro

the inherent craving for  
of energy need. L  
cause of the failure  
ponent, further fuels  
reward response n

Lastly, artificial sweeteners, precisely because they are sweet, encourage sugar craving and sugar dependence. Repeated exposure trains flavor preference

sucralose

neotame

1960

saccharin (in  
cyclamate (19



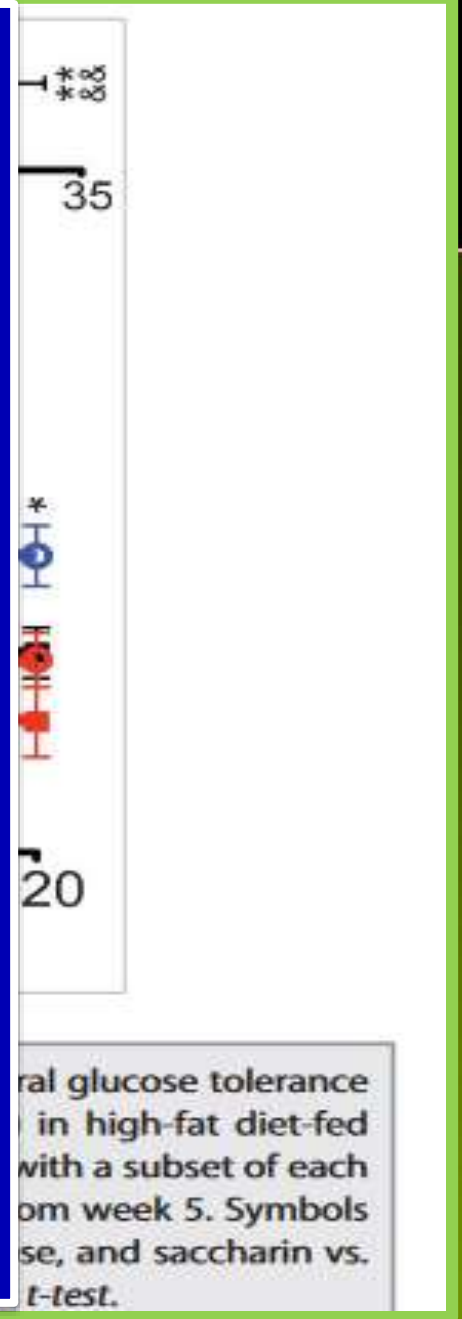


NAS consumption not only associates with various clinical parameters such as **BMI, blood pressure, HbA1 C% and fasting glucose levels**, but also with the presence of certain taxa, including expansion of the Actinobacteria phylum, the EnteroBacteriales order, and of various taxa from the Clostridiales order.

Non-calor

	Over-represented
	Under-represented
	Not determined / no
Palmnäs 2014	Asparta
Cowan 2013	Asparta
Rettig 2014	Sucralo
Abou-Donia 2008	Sucralo
Anderson 1980	Saccha
Daly 2013	Saccha
Suez 2014	Saccha

**Figure 1.** NAS-Bacteria in  
ing effects of NAS on me  
generation sequencing.







# Probiotics and Diet

**J Toxicol Environ Health. 1976 Nov;2(2):417-28.**

Effects of aspartame in young persons during weight reduction.

Knopp RH, Brandt K, Arky RA.

- No meaningful effect of weight reduction or aspartame was seen on plasma triglyceride and cholesterol, nor on any other parameter of hematologic, hepatic, or renal function that was measured.



# NAS- probiotics

**nature**  
International journal of science

Article | Published: 17 September 2014

## Artificial sweeteners induce glucose intolerance by altering the gut microbiota

Jotham Suez, Tal Korem, David Zeevi, Gili Zilberman-Schapira, Christoph A. Thaiss, Ori Maza, David Israeli, Niv Zmora, Shlomit Gilad, Adina Weinberger, Yael Kuperman, Alon Harmelin, Ilana Kolodkin-Gal, Hagit Shapiro, Zamir Halpern, Eran Segal ✉ & Eran Elinav ✉

- These NAS-mediated deleterious metabolic effects are abrogated by antibiotic treatment, and are fully transferrable to germ-free mice upon faecal transplantation of microbiota configurations from NAS-consuming mice, or of microbiota anaerobically incubated in the presence of NAS. We identify **NAS-altered microbial metabolic pathways that are linked to host susceptibility to metabolic disease, and demonstrate similar NAS-induced dysbiosis and glucose intolerance in healthy human subjects.**







# The portal to the GI Tract



**We know the problem-  
How do we prevent?**



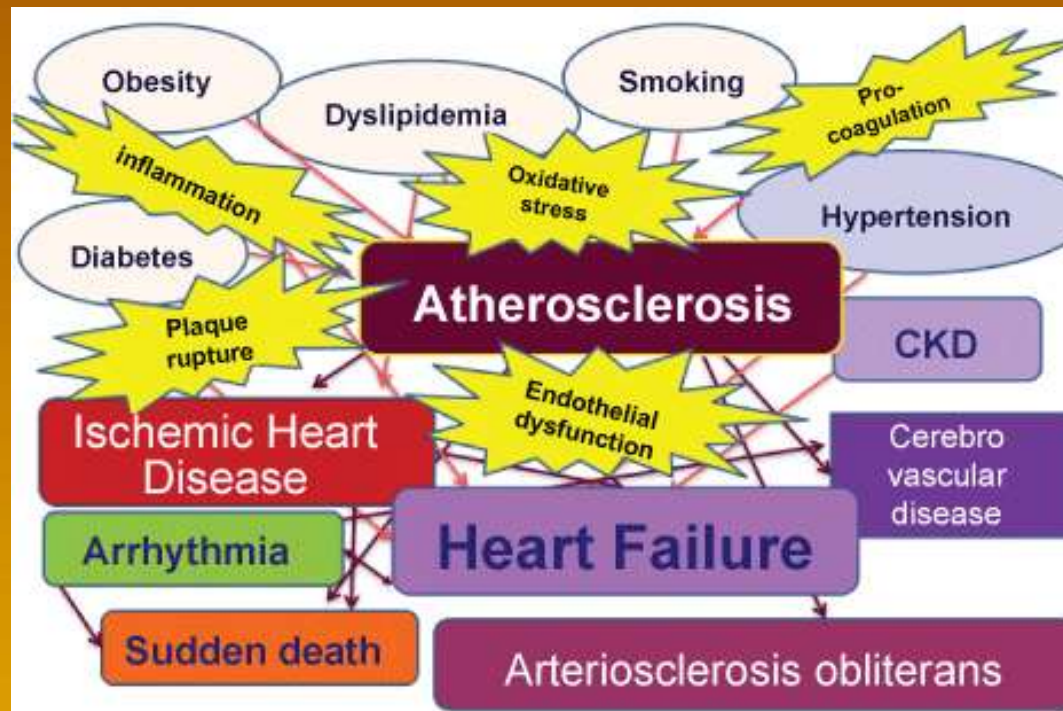
- “You pediatric dentists are the guardians to the portal of the gastro-intestinal tract”
- - Pediatric Gastroenterologist

**Romantic View- like Knights!**



# Probiotics and Microbiome

- Cardiovascular Disease





# Microbiome and Death

SCIENTIFIC REPORTS



Altmetric: 18

Article | [OPEN](#)

## Associations between Periodontal Microbiota and Death Rates

Chung-Jung Chiu , Min-Lee Chang & Allen Taylor

- These data suggested that specific combinations of periodontal bacteria, even without inducing clinically significant periodontitis, may have a significant impact on human cause-specific death rates. Our findings implied that increased disease and mortality risk could be transmittable via the transfer of oral microbiota, and that developing personalized strategies and maintaining healthy oral microbiota beyond protection against periodontitis would be important to manage the risk.

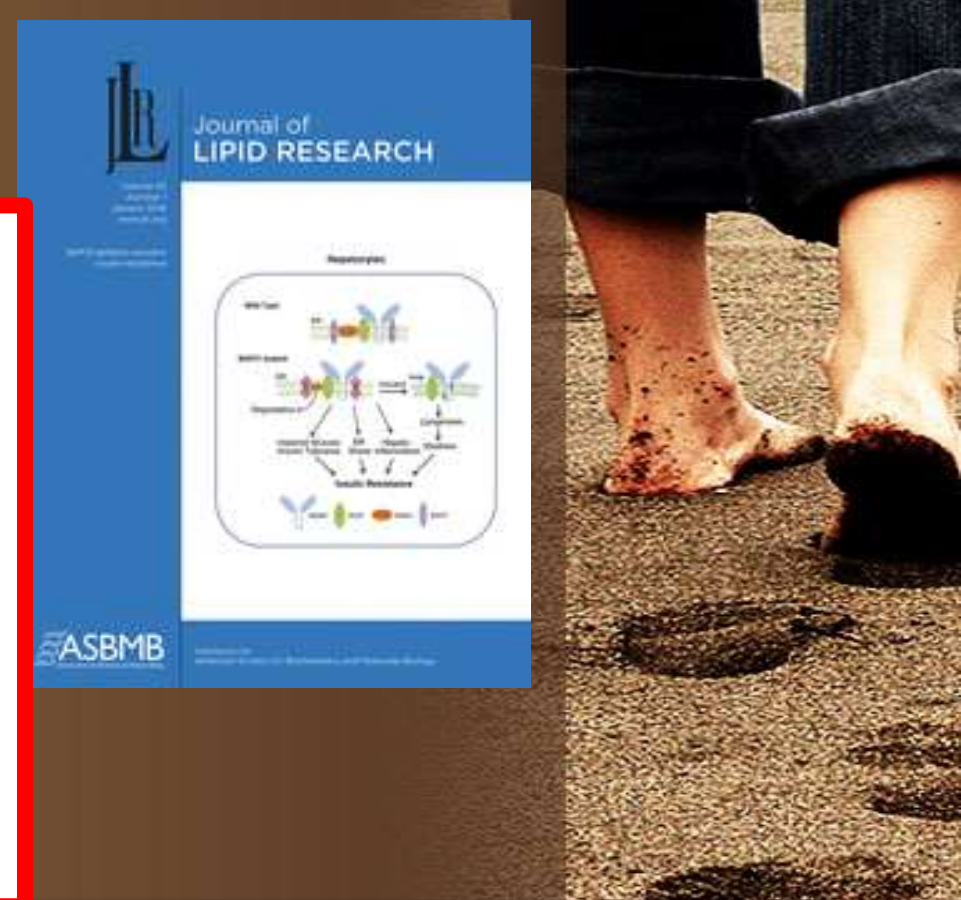




# Heart Disease and “Fries”

## Deposition and hydrolysis of serine dipeptide lipids of Bacteroidetes bacteria in human arteries: relationship to atherosclerosis

Reza Nemati<sup>1,\*</sup>, Christopher Dietz<sup>1,\*</sup>, Emily J. Anstadt<sup>†</sup>, Jorge Cervantes<sup>§</sup>, Yaling Liu<sup>\*\*</sup>, Floyd E. Dewhirst<sup>††</sup>, Robert B. Clark<sup>†</sup>, Sydney Finegold<sup>§§</sup>, James J. Gallagher<sup>\*\*\*</sup>, Michael B. Smith<sup>\*</sup>, Xudong Yao<sup>\*,†††</sup> and Frank C. Nichols<sup>2,\*\*</sup>



- These results suggest that commensal Bacteroidetes bacteria of the gut and the oral cavity may contribute to the pathogenesis of TLR2-dependent atherosclerosis through serine dipeptide lipid deposition and metabolism in artery walls.



# 7 years ago- it was missed

## RESEARCH ARTICLE

# Phosphorylated Dihydroceramides from Common Human Bacteria Are Recovered in Human Tissues

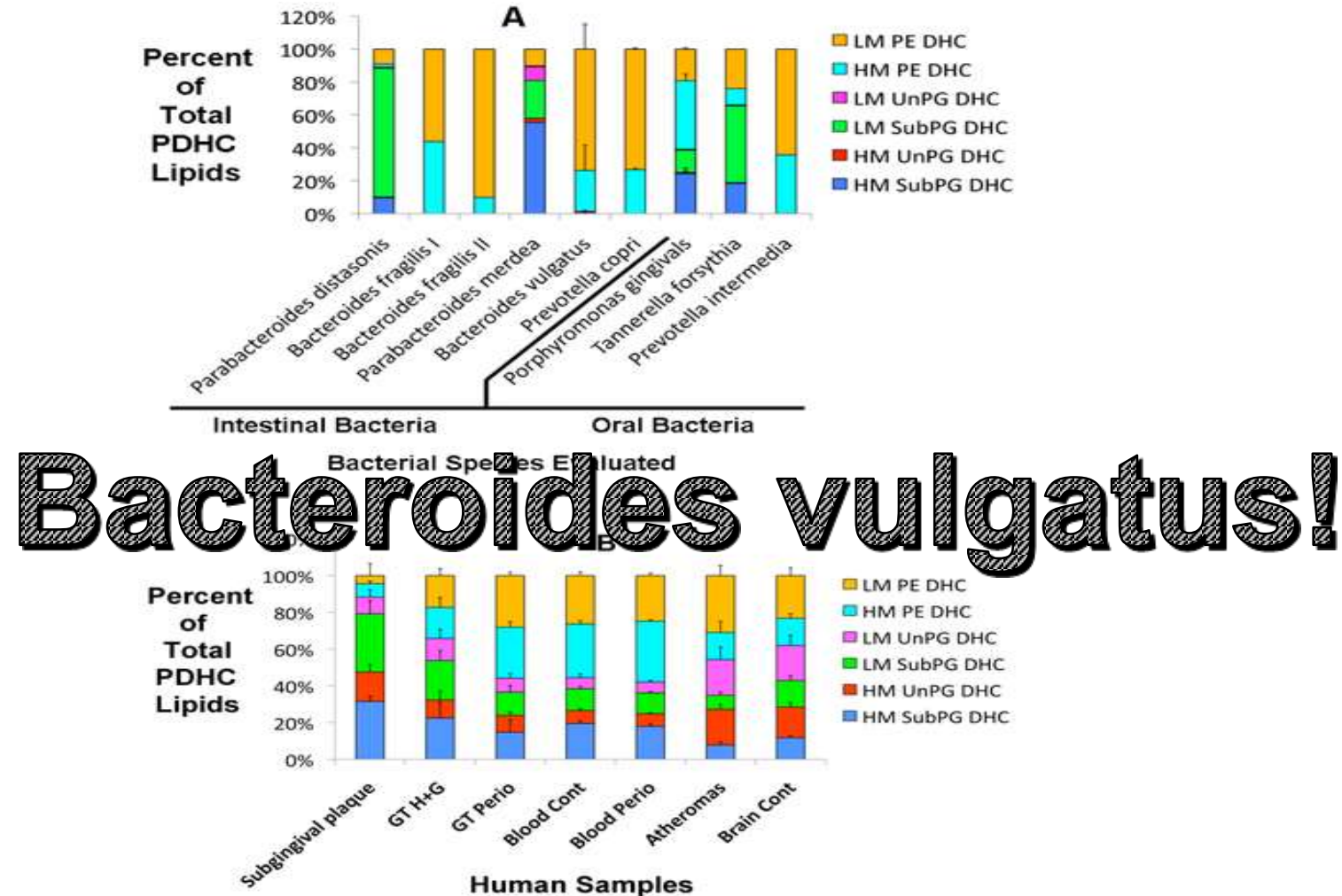
Frank C. Nichols , Xudong Yao, Bekim Bajrami, Julia Downes, Sydney M. Finegold, Erica Knee, James J. Gallagher, William J. Housley, Robert B. Clark

- We now report that synthesis of these lipids can be attributed to a small number of intestinal and oral organisms within the Bacteroides, Parabacteroides, Prevotella, Tannerella and Porphyromonas genera. **Additionally, the PDHCs are not only present in gingival tissues, but are also present in human blood, vasculature tissues and brain.** Finally, the distribution of these TLR2-activating lipids in human tissues varies with both the tissue site and disease status of the tissue suggesting a role for PDHCs in human disease.





**Figure 2. Recovery of bacterial phosphorylated dihydroceramides in intestinal and oral bacteria, subgingival plaque samples, blood plasma, atheroma and brain samples.**



Nichols FC, Yao X, Bajrami B, Downes J, Finegold SM, et al. (2011) Phosphorylated Dihydroceramides from Common Human Bacteria Are Recovered in Human Tissues. PLOS ONE 6(2): e16771.

<https://doi.org/10.1371/journal.pone.0016771>

<http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0016771>



# No guts, No glory! ACS

## Harvesting the microbiome of athletes

- Isolated unique strain of bacteria from fecal samples of marathon runners and is beginning to evaluate its properties. Determined that the bug excels at breaking down lactic acid in a test tube and remains viable after it passes through the digestive system of mice. The researchers are now feeding the bacteria to mice to measure its effects on lactic acid levels and fatigue. Another strain found in other athletes breaks down carbohydrates for energy in long distance runners.
- **Problem: Press releases and no published research**





# No guts, No glory! GUT

BMJ Journals

Gut



The microbiome of professional athletes differs from that of more sedentary subjects in composition and particularly at the functional metabolic level

- Athletes had relative increases in pathways (eg, amino acid and antibiotic biosynthesis and carbohydrate metabolism) and faecal metabolites (eg, microbial produced short-chain fatty acids (SCFAs) acetate, propionate and butyrate) associated with enhanced muscle turnover (fitness) and overall health when compared with control groups. **Differences in faecal microbiota between athletes and sedentary controls show even greater separation at the metagenomic and metabolomic than at compositional levels** and provide added insight into the diet–exercise–gut microbiota paradigm.



# No guts, No glory! GUT

Exercise and associated dietary extremes impact on gut microbial diversity **FREE**

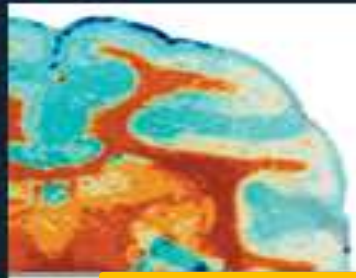
Siobhan F Clarke<sup>1, 2, 3</sup>, Eileen F Murphy<sup>2, 4</sup>, Orla O'Sullivan<sup>1</sup>, Alice J Lucey<sup>5</sup>, Margaret Humphreys<sup>6</sup>, Aileen Hogan<sup>2</sup>, Paula Hayes<sup>2</sup>, Maeve O'Reilly<sup>2, 4</sup>, Ian B Jeffery<sup>2, 3</sup>, Ruth Wood-Martin<sup>7</sup>, David M Kerins<sup>8, 9</sup>, Eamonn Quigley<sup>2</sup>, R Paul Ross<sup>1, 2</sup>, Paul W O'Toole<sup>3</sup>, Michael G Molloy<sup>10</sup>, Eanna Falvey<sup>10, 11</sup>, Fergus Shanahan<sup>2, 10, 12</sup>, Paul D Cotter<sup>1, 2</sup>

- As expected, athletes and controls differed significantly with respect to plasma creatine kinase (a marker of extreme exercise), and inflammatory and metabolic markers. **More importantly, athletes had a higher diversity of gut micro-organisms, representing 22 distinct phyla, which in turn positively correlated with protein consumption and creatine kinase.** The results provide evidence for a beneficial impact of exercise on gut microbiota diversity but also indicate that the relationship is complex and is related to accompanying dietary extremes.





# Propionic Neuroprotectant



The Official Journal of  
ISPNE INTERNATIONAL SOCIETY OF  
PSYCHONEUROENDOCRINOLOGY

Psychoneuroendocrinology

Probiotic treatment reduces depressive-like behaviour in rats independently of diet

Anders Abildgaard  , Betina Elfving, Marianne Hokland, Gregers Wegener, Sten Lund

- Probiotic therapy for anxiety depression

Independently of diet, probiotic treatment markedly reduced depressive-like behaviour in the forced swim test by 34% (95% CI: 22–44%). Furthermore, probiotic treatment skewed the cytokine production by stimulated blood mononuclear cells towards IFN $\gamma$ , IL2 and IL4 at the expense of TNF $\alpha$  and IL6. In addition, probiotics lowered hippocampal transcript levels of factors involved in HPA axis regulation (Crh-r1, Crh-r2 and Mr), whereas HFD increased these levels. **A non-targeted plasma metabolomics analysis revealed that probiotics raised the level of indole-3-propionic acid, a potential neuroprotective agent.**



# Propionic Neuroprotectant



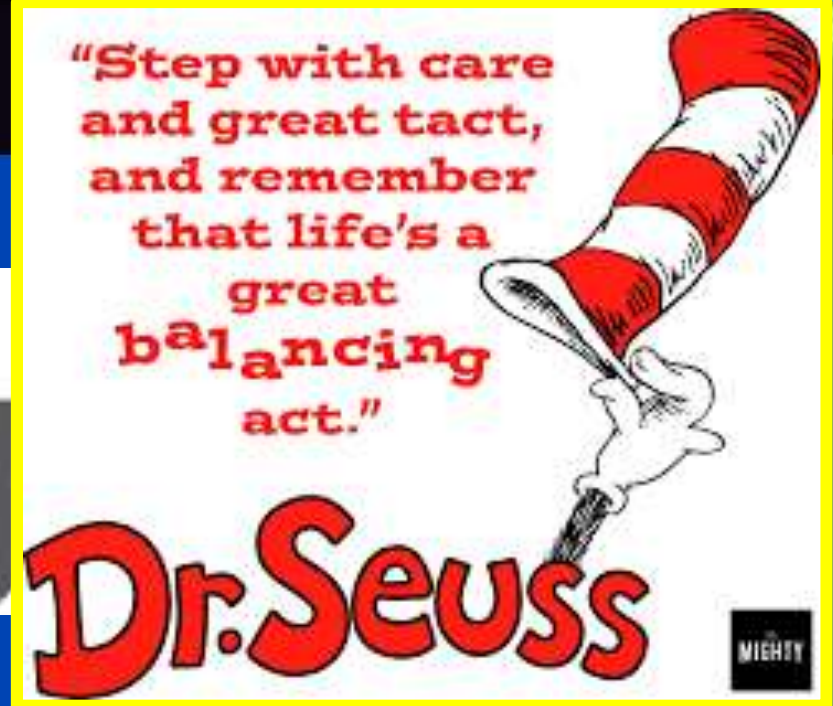
- **3-Indolepropionic acid (IPA), or indole-3-propionic acid, is a potent neuroprotective antioxidant and plant auxin that is being studied for therapeutic use in Alzheimer's disease.** It is endogenously produced by human microbiota and has only been detected in vivo when the species **Clostridium sporogenes** is present in the gastrointestinal tract. As of April 2016, **C. sporogenes**, which uses tryptophan to synthesize indole and subsequently IPA, is the only species of bacteria known to synthesize IPA in vivo at levels which are subsequently detectable in the blood plasma of the host.



# onic Neuroprotectant



balancing



- Balancing Act- removes indole and propionic-protects!!!
- *Clostridium sporogenes* inhibits action of *Clostridia boltae* and *histolyticum*



# IPA- plant hormone



## Metabolomics analysis reveals large effects of gut microflora on mammalian blood metabolites

William R. Wikoff, Andrew T. Anfora, Jun Liu, Peter G. Schultz, Scott A. Lesley, Eric C. Peters, and Gary Siuzdak

PNAS 2009 March, 106 (10) 3698-3703. <https://doi.org/10.1073/pnas.0812874106>



- Plasma extracts from germ-free mice were compared with samples from conventional (conv) animals by using various MS-based methods. The bacterial-mediated production of bioactive indole-containing metabolites derived from tryptophan such as indoxyl sulfate and the antioxidant indole-3-propionic acid (IPA) was impacted. Production of IPA was shown to be completely dependent on the presence of gut microflora and could be established by colonization with the bacterium *Clostridium sporogenes*.



# IPA and Diabetes...



## Indolepropionic acid and novel lipid metabolites are associated with a lower risk of type 2 diabetes in the Finnish Diabetes Prevention Study

Vanessa D. de Mello , Jussi Paananen, Jaana Lindström, Maria A. Lankinen, Lin Shi, Johanna Kuusisto, Jussi Pihlajamäki, Seppo Auriola, Marko Lehtonen, Olov Rolandsson, Ingvar A. Bergdahl, Elise Nordin, Pirjo Ilanne-Parikka, Sirkka Keinänen-Kiukaanniemi, Rikard Landberg, Johan G. Eriksson, Jaakko Tuomilehto, Kati Hanhineva  & Matti Uusitupa

*Scientific Reports* 7, Article number: 46337

Received: 03 October 2016

- Higher indolepropionic acid was associated with reduced likelihood of T2D in the DPS. Interestingly, in those who remained free of T2D, indolepropionic acid and various lipid species were associated with better insulin secretion and sensitivity, respectively.



# Probiotic vs Probiotic



Research in Microbiology

Volume 159, Issue 6, July–August 2008, Pages 470–475



Surface-bound proteins of *Lactobacillus plantarum* 423 that contribute to adhesion of Caco-2 cells and their role in competitive exclusion and displacement of *Clostridium sporogenes* and *Enterococcus faecalis*

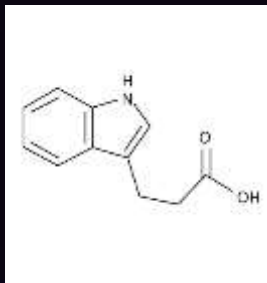
Kamini Ramiah , Carol A. van Reenen , Leon M.T. Dicks 

- Colonization of **L. plantarum** 423 to Caco-2 cells prevented adhesion of 74% of cells of **C. sporogenes** LMG 13570 and 62% of cells of *E. faecalis* LMG 13566. Furthermore, *L. plantarum* 423 displaced 81% of cells of *C. sporogenes* LMG 13570 and 91% of cells of *E. faecalis* LMG 13566 from Caco-2 cells. **L. plantarum 423 is a potential probiotic strain.**

The Caco-2 cell line is a continuous cell of heterogeneous human epithelial colorectal adenocarcinoma cells, developed by the Sloan-Kettering Institute for Cancer Research through research conducted by Dr. Jorgen Fogh.



# Tree of Life- evolved!



"Most people hate the idea of working

e that  
metab  
h cou

## Indole-3-propionic acid (IPA)

Synonyms: 3-(3-Indolyl)propanoic acid, 3-Indolepropionic acid

Plant hormone with numerous cell growth functions including cell division, elongation, autonomic loss of leaves, and the formation of buds, roots, flowers, and fruit

Compound is inhibited by light, which plays an important role in aiding the plant grow toward light sources

Appearance: yellow to orange to brown powder

Melting Point: 134-135 °C

Soluble in ethanol



# Take Home- probiotics

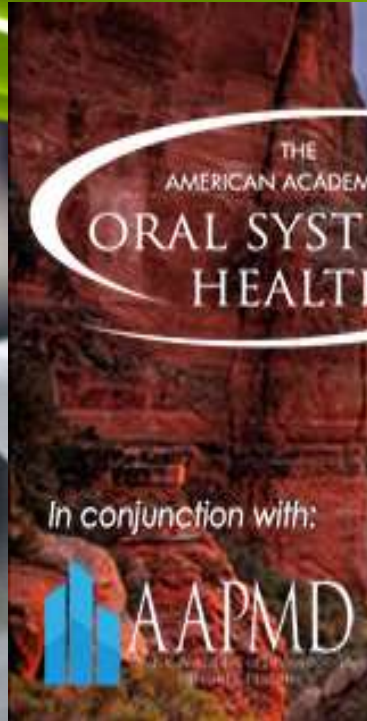


- Periodontal disease and cardiovascular pathology are amenable to prevention with probiotics
- Both occur due to lack of protective microbes



# Le

## A Lot



dentistry  
Health  
Open  
ember 8-10, 2018  
t | Las Vegas, NV