

Probiotics to prevent NEC: what is the evidence?

NASPGHAN Annual Meeting

Friday October 24, 2014

Atlanta, GA

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Disclosures

I have the following financial relationships to disclose:

*Lallemand Human Nutrition
(research contract)

*Abbott Nutrition (honorarium)

*Mead Johnson Nutrition (honorarium)

*Nestlé Nutrition (honorarium)

*Procter & Gamble (honorarium)

Antib Therapeutics (stockholder)

* Products or services produced by this company are relevant to my presentation.

Learning objectives

1. Provide an update on the composition of the gut microbiota in early life.
2. Consider the impacts of an altered microbiota.
3. Critically assess the evidence for using probiotics to prevent necrotizing enterocolitis.



Thomas Abrahamsson
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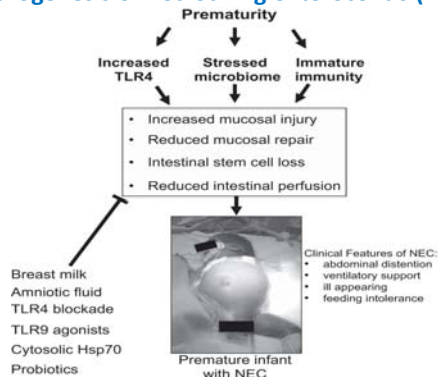
Case presentation

Case #1. 5-day-old M born by C-section @ 32 weeks, 1,000 g about to start on enteral formula feedings post r/o sepsis & course of IV antibiotics.

How can one reduce the risk of necrotizing enterocolitis?:

- a) Probiotics
- b) Oral antibiotics
- c) Prebiotics
- d) Gradual introduction of enteral feedings, breast milk, donor milk
- e) Fecal microbial transplant

Pathogenesis of necrotizing enterocolitis (NEC)



P. Lu et al. Am J Physiol 2014;306:G917-G928

Differing levels of analyses of the gut microbiome

Meta-omics	Molecule	Knowledge	Limits	Clinical implications
Phylogeny	sIS-DNA	• Bacterial composition & diversity	• No information on bacterial functions • Except archaea	• Composition dysbiosis • Healthy or disease specific species
Metagenomics	Chromosomal genomic DNA	• High resolution • Microbiome profiling • Genes contents from uncultivated microbes	• No information on microbial expressed functions	• Functional dysbiosis • Healthy or disease specific microbial genes • Targeted diagnostics • Functional based studies
Metatranscriptomics	Messenger RNA/ cDNA	• High resolution gene expression profiling • Differential microbial gene expression various physiological/ environmental conditions	• Poor stability of bacterial mRNA • Representativity unknown (Multiple purification steps needed) • No unique protocol	• Functional Dysbiosis • Microbial activity kinetics • Expressed genes at specific time and location • Specific monitoring of active bacteria
Metaproteomics	Proteins/ Peptides	• High resolution protein mapping and profiling • Differential microbial proteins production under various physiological/ environmental conditions	• Many unknown proteins in databases • Heterogeneous stability • No unique protocol	• Function confirmed genome annotation improvement • Eukaryotes-procaryotes analog identification • Biomarkers
Metabolomics	Metabolites	• Microbial and host metabolic profiling • Easy to perform on very low amount of material (nanogram range)	• Many unknown metabolites in databases • Strict identification of compound families • No unique protocol • Combination of host and microbial molecules	• New pathways confirmed or identified • High throughput metabolomic screening of biomarkers • Easy translation to clinical setting

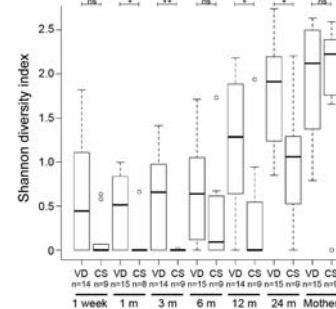
P Lepage, et al. Gut 2013;62:146-158

Development of the gut microbiota

- Fetal intestine: “sterile”
- Initial colonization determined by:
 - Delivery mode (caesarian section vs. vaginal)
 - Diet (breast feeding vs. formula feedings)
 - Hygiene (exposure to pathogens)
 - Medication (antibiotics)
- Temporal changes over the first years of life

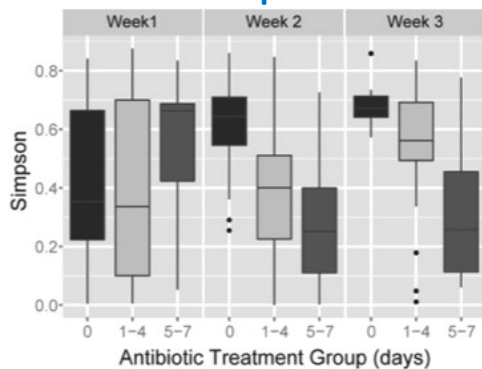
S Rautava, et al. *Nat Rev Gastroenterol* 2012;9:565-576
 F Backhed, et al. *Cell Host & Microbes* 2012;12:611-622
 M-E Sanders, et al. *Gut* 2013;62:787-796

Gut diversity in 15 vaginally delivered and 9 caesarian section infants



HE Jakobsson, TR Abrahamsson, et al. *Gut* 2014;63:559-566

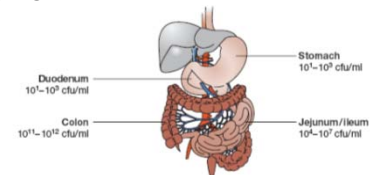
Antibiotic use in preterm infants



C. Greenwood et al. *J Pediatr* 2014;165:23-29

Distribution of microbes in the gut

- Present in all parts of the intestinal tract
- Increase from esophagus to colon
 - acid production
 - bile
 - motility
 - ileocecal valve



- Surface-lumen axis: more anaerobes in the outer mucus
- FISH: bacteria are not in direct contact with the mucosa
 - at least, in healthy subjects (vs. Crohn disease)

Impact of the gut microbiota on human health.
 JC Clemente, et al. *Cell* 2012;148:1258-70

Reduced bacterial diversity (dysbiosis): an emerging theme across diseases

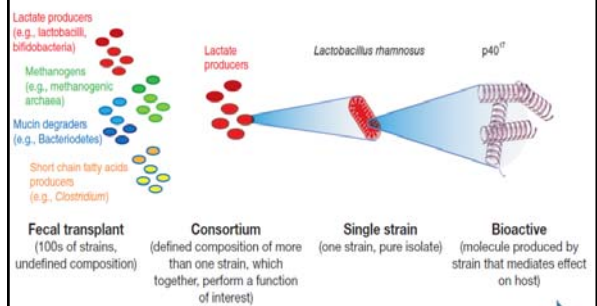
- Microbiota affected by:
 - Infections
 - Antibiotics
 - Xenobiotics
- Diabetes mellitus
- Obesity
- Cancers: gastric, colonic
- Inflammatory bowel diseases
- Irritable bowel syndrome
- Necrotizing enterocolitis



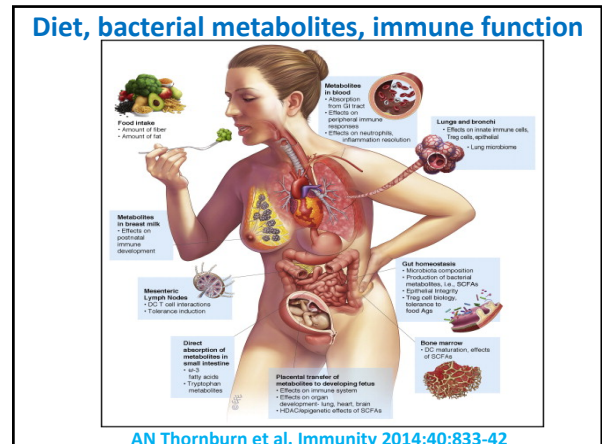
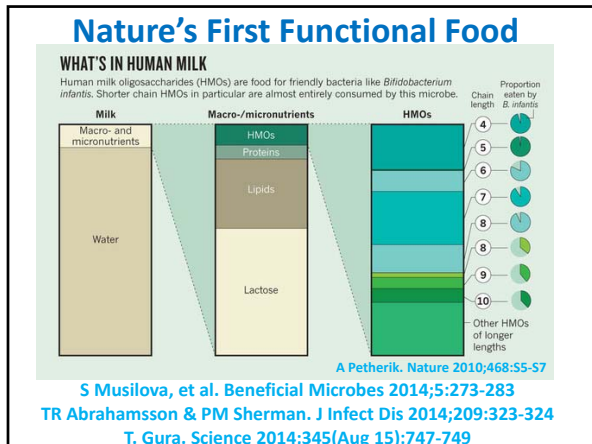
Scientific American
 June 2012

C Peterson & JL Round. *Cell Microbiol* 2014;16(7):1024-1033

How does one increase diversity?



B. Olle. *Nat Biotechnol* 2013;31:309-315

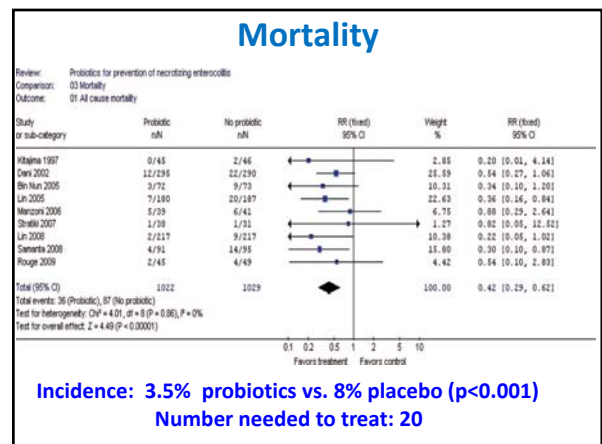
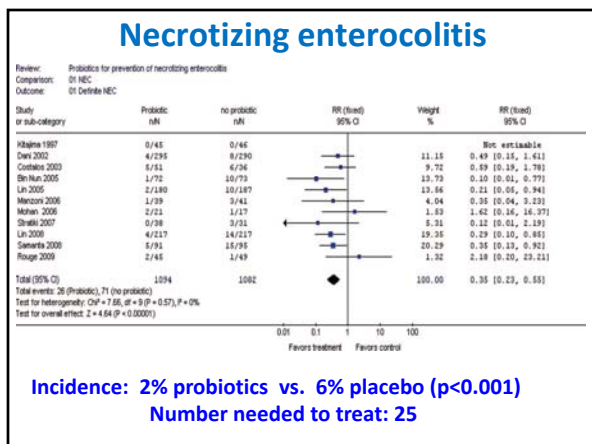


Definition & examples of a probiotic

Is:	Is not:	Examples:
Microbe	Synonymous with "commensal"	<i>Bifidobacterium (longum, bifidum)</i>
Alive	Synonymous with "live, active culture"	<i>Streptococcus thermophilus</i>
Defined and properly named	Live vaccine	<i>Lactobacillus (GG, acidophilus, rhamnosus, casei, plantarium)</i>
Safe	Fecal enema	<i>Lactococcus (lactis, cremoris)</i>
Regulatory categories		<i>Escherichia coli (Nissle 1917)</i>
- Food - Dietary supplement - Drug - Designer/genetically modified - Direct fed (animal uses)		<i>Saccharomyces (boulardii, cerevisiae)</i>

C. Hill et al. *Nat Rev Gastroenterol Hepatol* 2014;11(8):506-514

- ## Meta-analyses of probiotics to prevent NEC
- Study design:**
- **Birth weight:** includes <1,500 g (VLBW infants)
 - **Randomized**
 - **Double-blinded with placebo:** only in 2 (and both were negative trials!)
 - **Dose:** 0.5- 5 x 10⁹ bacteria/day
 - **Treatment duration:** started on day 1-7 & stopped at 4 weeks of age or hospital discharge
 - **Probiotic strains:** different strains/combinations in all trials, but two (LGG, both were negative!)
 - **Breast milk exclusive:** none (poorly described)
- Deshpande et al. *Pediatrics* 2010;125:921-30
W. Mihatsch. *Clin Nutr.* 2012;31:6-15
Q Wang, et al. *J Pediatr Surg* 2012;47:241-8



Use probiotics to prevent NEC?

“Evidence that probiotics reduce mortality is as conclusive as that for surfactant for RDS.”

WO Tarnow-Mordi, et al. *Pediatrics* 2010;125:1068-70

“Great reason to be hopeful . . . However, meta-analyses and multiple small trials have led us astray before”

R Soll. *Pediatrics* 2010,125:1071-2

“We suggest that the effect of probiotics on the incidence of NEC is still controversial.”

MY Oncel et al. *J Pediatr* 2014;165:417

“The efficacy of probiotics is no longer questionable. They are more firmly established than almost any other therapy in Neonatology.”

KJ Barrington, *J Pediatr* 2014;165:417-418

ProPrem trial

- 10 NICU's in Australia + New Zealand
- 1,099 VLBW infants (<1500g, <32 wk ga)
- Double-blinded, placebo-controlled
B. infantis DSM 96579 +
B. animalis subspecies *lactis* DSM 15954 +
S. thermophilus DSM 15957
 (1 X 10⁹/d)
- Repeat of a previous design (*Bin-Nun A, J Pediatr.* 2005;147:192-6.)
- 97% received breast milk - due to donor milk bank
- Low background incidence of NEC (4-5%)



SE Jacobs et al., *Pediatrics* 2013;132:1055-1062

Results of ProPrem trial

	Probiotics (n=548) n (%)	Placebo (n=551) n (%)	Risk Reduction:
NEC:	11 (2.0)	24 (4.4)	0.46 (0.23-0.93)
>1000g	1 (0.3)	10 (3.2)	NNT = 43
<1000g	10 (4.3)	14 (5.9)	
Sepsis:	62 (13.1)	89 (16.2)	0.81 (0.61-8.08)
Mortality:	27 (4.9)	28 (5.1)	0.97 (0.60-1.58)

German Neonatal Observational Network: Decreased NEC and mortality, but not sepsis
 N=5,351
 C Hartel et al. *J Pediatr* 2014;165:285-9

Current view on probiotics to prevent NEC

Need studies of sufficient power in the ELBW (<1,000 g)

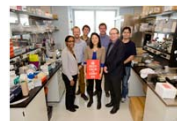
Confirm results of effective probiotic strain(s)

Double-blinded, as well as placebo-controlled

North American & western European context

Manufacturing process very important=quality!

TR Abrahamsson et al. *J Pediatr*, 2014; 165:389-394



Challenges related to probiotic use

- **Stability of formulations**
- **Dosage and timing of delivery**
- **Single versus combination strains**
- **Distraction from mother & donor milk access**
- **Safety concerns:**
 - for highly atopic subjects, cow's milk protein in some commercial probiotic preparations
 - bacteremia and fungemia with short gut s. & central line
 - mesenteric ischemia with severe illness, high dose, and multiple probiotic agents (in adults with acute pancreatitis)
 - severe immunodeficiency
 - extreme prematurity

M-E Sanders, et al. *Gut Microbes* 2010; 1:164-185

Case presentation revisited

Case #1. 5-day-old born by C-section @ 32 weeks, 1,000 g who is about to start on enteral formula feedings post r/o sepsis.

How can one reduce the risk of necrotizing enterocolitis?:

- Probiotics - in Asia-Pacific and parts of Europe**
- Oral antibiotics
- Prebiotics - require further study . . .
- Gradual introduction of GI feeding [mother's milk, milk bank (Pasteurized)] - in USA and Western Europe**
- Fecal microbial transplant

Take home messages in 2014:

Gut microbiota is increasingly recognized to play a role in promoting health.

Intestinal dysbiosis appears to play a role in various disease states, including NEC.

Probiotics: comparative efficacy and relative safety profiles are needed.

“Physicians should advocate for further research to define which strains and dose of probiotics should be used in specific conditions.”
Can Pediatr Soc Position Statement on Probiotics - Dec 3, 2012

Thank you for your attention!

Questions, comments, feedback...



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