Slide 1

Breast Milk, Gut Microbiome and disorders in early Childhood

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Eosinophilic Esophagitis – Why?

- gut microbiota as a result of changes in diet;
- antibiotic exposure;
- increased cesarean deliveries;
- Increased exposure to environmental allergens; changes in how food is grown, processed, and packaged;
- decreased prevalence of *Helicobacter pylori*; and
- reduced exposure to microbial disease (the hygiene hypothesis) in developed countries

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Elevated odds of EoE in North Carolina:

- antibiotic administration during the first year of life (6 fold higher risk)
- a trend toward increased
  - odds for cesarean delivery,
  - preterm birth, and
  - not having exclusive breast-feeding

exclusive breast-feeding was lowest in the EoE group (6%) compared with both GERD and Controls (23% and 19%, respectively).

- J Pediatr Gastroenterol Nutr. 2013 Jul;57(1):67-71.

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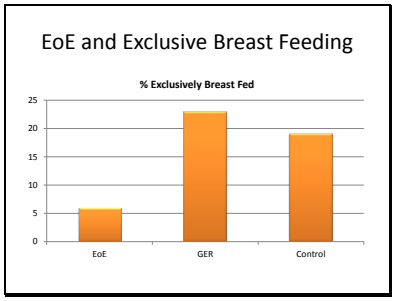

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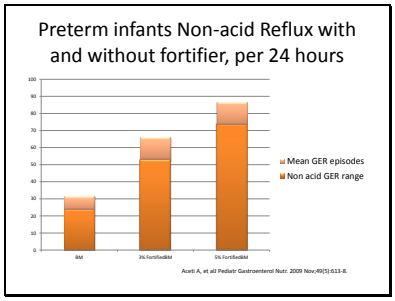

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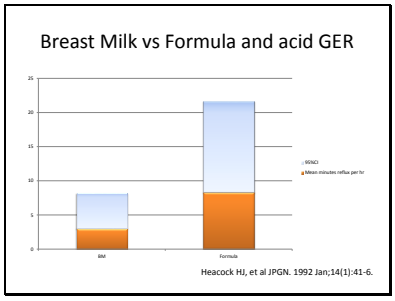

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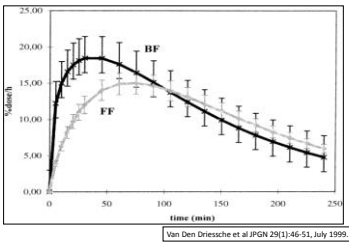
Gastric-emptying curves of breast-fed (BF) infants and formula-fed (FF) infants.



Van Den Driessche et al JPGN 29(1):46-51, July 1999.

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Breast milk may be a protective factor in GER

- enhances gastric emptying, and
- regurgitation and vomiting are symptoms of milk protein allergy, which is 5 to 10 times more frequent in formula fed than in breastfed infants
- However, recent studies suggest BM allows earlier resolution of reflux than formula fed by 1 month, but reflux amount per day is the same.

Campanozzi A, et al. Prevalence and natural history of gastroesophageal reflux: pediatric prospective survey. Pediatrics. 2009;123:779–783

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Neonatal Dysphagia and GER

- Prolonged feeding duration was significantly associated with decreased total, nonacid GER ($P < .03$).
- Significant positive correlations ($P < .05$) were detected between feeding flow rate vs frequency of GER
- Significant positive correlation ($P = .002$) was noted between feeding volume and clearance of reflux
- No difference between fortified BM and formula

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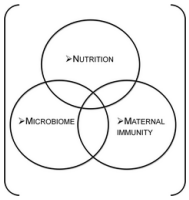
The gut mucosal immune system in the neonatal period



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Microbiome

- Human microbiome studies have demonstrated dynamic changes in bacterial composition in the gut during pregnancy and childhood development

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Microbiome in Disease

presence of pathogenic species, or absence of beneficial species, in early childhood has been suggested to play a key role in the

- initiation of preterm birth,
- asthma
- eczema
- allergy,
- Autism
- other immunological deficiency

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Fetal Microbiome?

- Historically, the fetus, as well as the intrauterine environment, has been considered sterile, with the initial microbial exposure taking place at birth vaginally or via C-section through contacting maternal vaginal or skin microbiota, respectively

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Fetal Microbiome?

- microbes in amniotic fluid
- umbilical cord blood
- meconium
- placental
- fetal membranes
- Suggest mother-to-baby efflux of commensal microbes may occur prior to birth

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- Commensal microbes may contribute fundamentally to infant and childhood development and immunity
- Few studies have determined the microbial composition of the first intestinal discharge, or meconium, in premature
- linked its bacterial content to maternal eczema and infant mucus congestion during the first year of life

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- In a randomized study, breastfed infants tended to have lower levels of potentially pathogenic Clostridium difficile than their formula-fed counterparts, who also tended to have had higher proportions of Bacteroides and Prevotella
- Although healthy infants often carry C. difficile asymptomatically in their gut in early infancy, its presence can alter community composition .

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BM

- Breastfeeding is associated with a lower risk of childhood and adult-onset obesity . This may be due, in part, to the effects of breastfeeding on the development of the microbiome, as early diet guides colonization
- Bacteria possess varying abilities to extract nutrients and energy from food; consequently, the microbiome can shift an infant's energy storage potential

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Maternal breastmilk is considered the gold standard for infant feeding

The clinical benefits of breastfeeding are wide ranging and appear to persist beyond weaning. A recent meta-analysis found robust evidence that breastfeeding reduces the incidence of

1. gastrointestinal infection,
2. respiratory tract infection,
3. otitis media,
4. sudden infant death syndrome (SIDS),
5. type I and type II diabetes mellitus,
6. atopic dermatitis,
7. asthma and
8. obesity

Additionally, for preterm infants, breastfeeding is vitally important in reducing the incidence of NEC.

*Ip S, Chung M, Raman G, Trikalinos TA, Lau J. A summary of the Agency for Healthcare Research and Quality's evidence report on breastfeeding in developed countries. Breastfeed Med 2009: **4** (Suppl. 1): S17–30.*

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- Further, oligosaccharides in breastmilk can selectively promote Bifidobacterium growth in the gut, shown by combinatorial genomic and culture approaches with parallel glycoprofiling
- A study of 56 mother–infant pairs found that high maternal BMI during pregnancy is associated with lower levels of key immunomodulators in breastmilk and infant gut Bifidobacterium counts , which may in turn contribute to long-term health and weight management in breastfed infants

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The mechanism of action of breastmilk remains poorly understood .
Composition of the infant microbiome is strongly implicated.
Breastfed infants harbour beneficial or 'friendly' bacteria such as *Bifidobacterium*, whilst formula-fed infants are more likely to host pathogenic bacteria such as *Escherichia coli*.
Breastmilk displays microbicidal properties through provision of
IgA,
IgM, l
ysozyme,
lactoferrin and
complement. The mechanisms of the anti-inflammatory and immunomodulatory effects are less clear, but certainly growth factors and cytokines appear to play a role: these include
epidermal growth factor (EGF),
IL-10,
TGFβ,
sCD14 and
IFN

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- A study of 30 children, enrolled in an ongoing longitudinal study, found that at age 10 overweight children had lower levels of gut Bifidobacterium as infants, compared with their normal-weight counterparts However, epidemiological longitudinal studies assessing the microbiome–obesity relation are lacking

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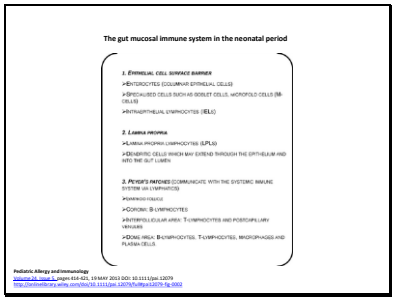

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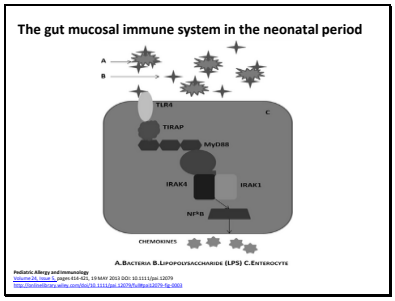

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Allergy

- In studies of infants, decreased microbial diversity in the first weeks of life was related to risk of allergy and atopy in infancy and at school age.
- Correspondingly, in a study of infants exposed to antibiotics, gut microbes appeared to influence the maturation of T helper cells (Th1) immune responses, $CD4^+$ T-cell phenotype, Th1/Th2/Th17 development and activity, and regulatory T-cell function

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Allergy

- Targeted investigation of neonatal microbial colonization patterns with Bifidobacterium found associations between enhanced maturation of protective mucosal immunoglobulins and early intense colonization with Bacteroides fragilis might downregulate immune responsiveness in infancy

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BM, Microbiome and Allergy

- Moreover, a novel evaluation of diet-dependent interactions within the relationship between the microbiome and host transcriptome identified not only differences in specific bacteriology between breastmilk and formula exposed infants by 3 months, but also metabolic function, immunity, and defense genes, which were more readily upregulated in the breastfed infants

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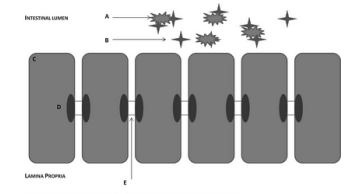

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- A novel study of the host transcriptome and microbiome simultaneously in infants, highlighting differences in breastfed and formula-fed infants via gut colonization, through host expression of genes associated with the innate immune system

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Intestinal function	Importance in mature gut	Importance in infant gut
Peristaltic movements	Expulsion of bacteria in the stool.	Generalized reduced peristalsis in preterm infant gut.
Mucous secretion	Mucous secreted by goblet cells acts as a physical barrier against pathogens by preventing bacterial adherence. It also contains immunologically active immunoglobulin A (IgA).	Preterm infants may be mucin deficient as the mucin gene is only fully expressed between 23 and 27 weeks gestation [52].

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Intestinal function	Importance in mature gut	Importance in infant gut
Peptide secretion	Lysozymes and defensins are secreted by Paneth cells in response to microbes. They affect the number and composition of the colonizing microbiota [53].	The preterm gut has fewer Paneth cells and reduced expression of alpha-defensin [56]. In mice, cathelin-related antimicrobial peptide (CRAMP) is observed to protect against Listeria monocytogenes in the first 2 weeks of life. CRAMP expression may contribute to normal bacterial colonization and protect against infection in the post-natal period [55].
Microbial recognition	Epithelial and dendritic cells 'sample' the gut microbiota by detecting pathogen-associated molecular patterns (PAMPs), for example, lipopolysaccharide (LPS) and lipoteichoic acid (LTA). Epithelial cells express NOD-like receptors (NLR) and Toll-like receptors 2 and 4 (TLR-2 and TLR-4) that detect LPS and LTA [58]. Ligation of TLRs and NLRs in the gut can produce a localized response (whereas elsewhere in the body this usually activates the innate immune response).	TLR-2 and TLR-4 are active in foetal gut at 18–21 weeks gestation and are thought to be important in infants [57]. TLR-4 expression may play a role in necrotizing enterocolitis (NEC) [54, 59].

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Transepithelial antigen transport	Antigen presentation to lymphocytes is undertaken by specialized microfold cells (M cells) and dendritic cells. Enterocytes express MHC class II and may also act as antigen-presenting cells (APCs) [7].	In newborns, the neonatal Fc receptor (FcRn) mediates bidirectional transport of IgG across the epithelium [60].
Interface to the systemic immune system	Efficacy of mucosal vaccines given orally such as the polio vaccine, which induces systemic immunity [65].	Needle-free mucosal vaccines are an attractive option for infants; however, infant IFNg responses to oral polio vaccine appear attenuated. Defective IFNg production may predispose infants to infection by intracellular pathogen [66].

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Prevention of bacterial translocation	Tight junctions The permeability of the intestine to microbes is dependent on the integrity of 'tight junctions' between enterocytes. Tight junctions are composed of a ZO-1 protein within each enterocyte, which are linked together by claudins (Fig. 1). TLR signalling	Presence of LPS in the bloodstream of infants with NEC is high at onset of the illness implicating intestinal barrier failure as an early factor in the pathogenesis [63]. A leaky intestinal barrier allows bacterial products and proinflammatory cytokines into the bloodstream to exert effects at organ sites, which can result in multi-organ failure [64].

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Factors associated with development of NEC

- Prematurity
- Formula feeding
- Abnormal intestinal colonisation
- Bacterial translocation
- Mode of delivery
- Intestinal tissue hypoxia

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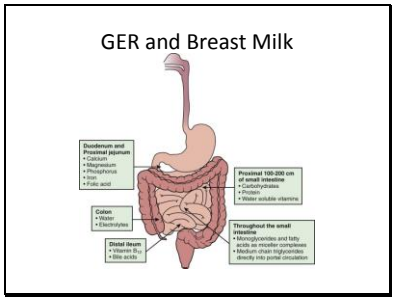

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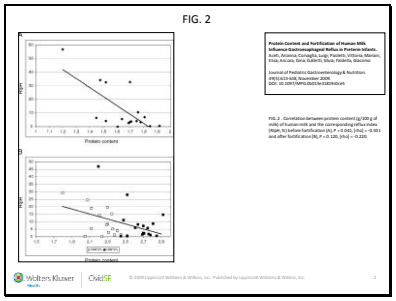

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