Gut Health Unveiled: Understanding Prebiotics, Probiotics, and Synbiotics

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Disclosures

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The opinions reflected in this presentation are those of the speaker and independent of Nutricia North America

Objectives

1. Highlight the differences and relationships between prebiotics, probiotics, synbiotics, and the gut microbiota.
2. Discuss the functionality and research behind B. breve M-16V.
3. Explain the role of FOS & GOS as a prebiotic and its impact on digestive health.
4. Discuss the potential health benefits of prebiotics, probiotics, and synbiotics in the allergic population

Highlight the differences and relationships between prebiotics, probiotics, synbiotics, and the gut microbiota.

A healthy gut or a "diverse" gut?

Gut microbiota: highly diverse and harboring trillions of microorganisms in human digestive system

- **Gut Microbiota changes dramatically over the first 3 years of life:**
- developmental phase (months 3–14)
- transitional phase (months 15–30)
- stable phase (months 31–46)

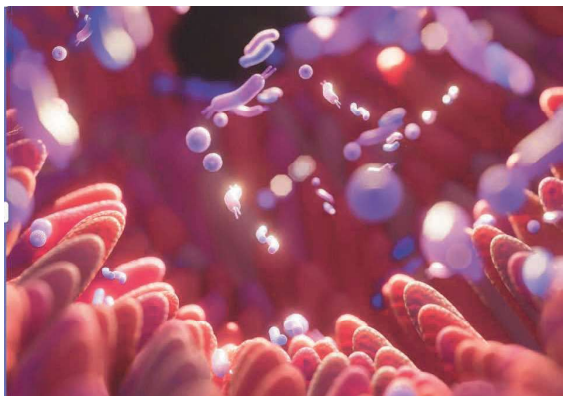
Dysbiosis: loss of balance in gut microbiota may arise in some specific situations

Forsberg et al. Exp Allergy. 2016;46(12):1506-1521; Moos W, et al. Biores. 2017; 6(1): 46..

Role of microbiota on immune system

The human gut microbiome modulate the immune system in many ways but two important factors are:

- Production of bacterial metabolites such as SCFA (e.g. butyrate)
- Supporting the epithelial and mucosal barrier
- Butyrate:
  - Maintains intestinal barrier: tight junction proteins and mucus production
  - Acts as source of energy for colonocytes
  - Expands regulatory T cells and increases IgG and IgA levels
  - Increases the T-regulatory cytokines IL-10 and TGF-B



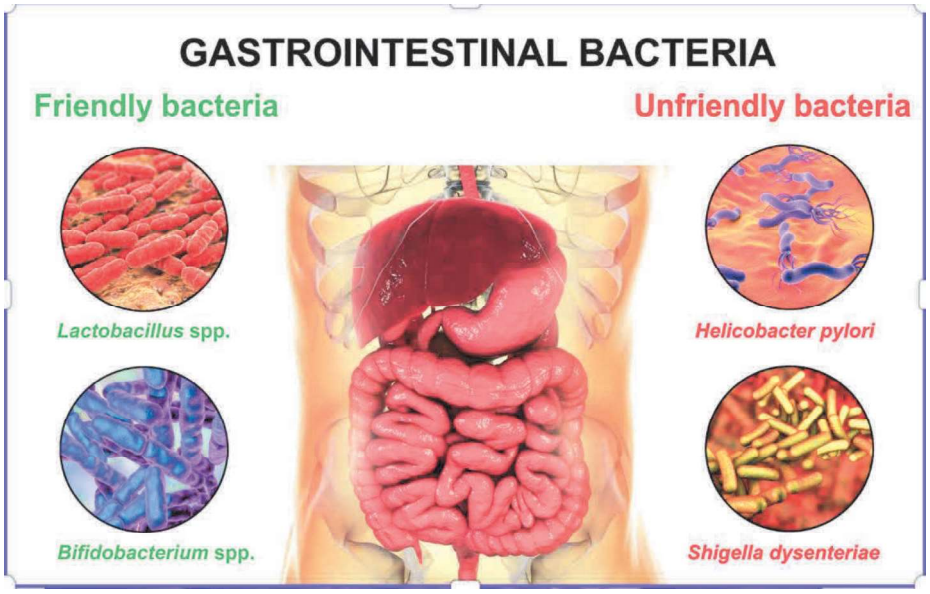
Adami et al. Yale J Biol 2016; 89(3): 309–324.; Thorburn et al. Nature Communications 2015; 23:6:7320.

Early Life Microbiome

Important butyrate producing bacteria:

Bifidobacteria: ”assist” with butyrate production

Firmicutes phylum e.g. Lactobacillus, and in particular Clostridial clusters IV and XIVa. (not present in first 6 months)



Riviere et al. Front Microbiol. 2016;7:979; Sasaki et al. Allergy.2024 Jul;79(7):1789-1811.

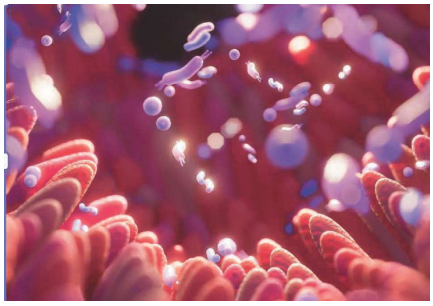
The International Scientific Association for Probiotics and Prebiotics (ISAPP)

- **Prebiotics:** substrate that is selectively utilized by host microorganisms conferring a health benefit
- **Probiotics**: live microorganisms that, when administered in adequate amounts, confer a health benefit on the host
- **Synbiotics:** synbiotics are a mixture comprising live microorganisms and substrate(s) selectively utilised by host microorganisms that confer a health benefit on the host.
- Two subsets:
  - A ‘synergistic synbiotic’ is a synbiotic in which the substrate is designed to be selectively utilized by the co-administered microorganism(s).
  - A ‘complementary synbiotic’ is a synbiotic composed of a probiotic combined with a prebiotic, which is designed to target autochthonous microorganisms.

Gibson et al. Nat. Rev. Gastroenterol. Hepatol. 2017;14:491–502.; Hill et al. Nat. Rev. Gastroenterol. Hepatol. 2014;11:506–514; Swanson et al. Nat Rev Gastroenterol Hepatol. 2020 Aug 21;17(11):687–701.

Explore data on the gut microbiota composition of children with food allergy and associations with health outcomes (e.g. infections)

Gut barrier



- Food intake, the microbiome and the gut barrier are highly connected and both the gut microbiome and gut barrier function are affected by food intake.
- Allergen absorption is increased when the gut barrier is disrupted.
- Barrier function impairment has been shown with participants with IgE-mediated allergy who were consuming the allergen.
- An intact gut barrier reduce allergen intake.
- The gut barrier Integrity depends on: mucus layer, **gut microbiome**, tight junctions, anti-microbial peptides etc. and both the innate/adaptive immune.

Zhang Int J Mol Sci. 2022 Sep; 23(17): 9588.

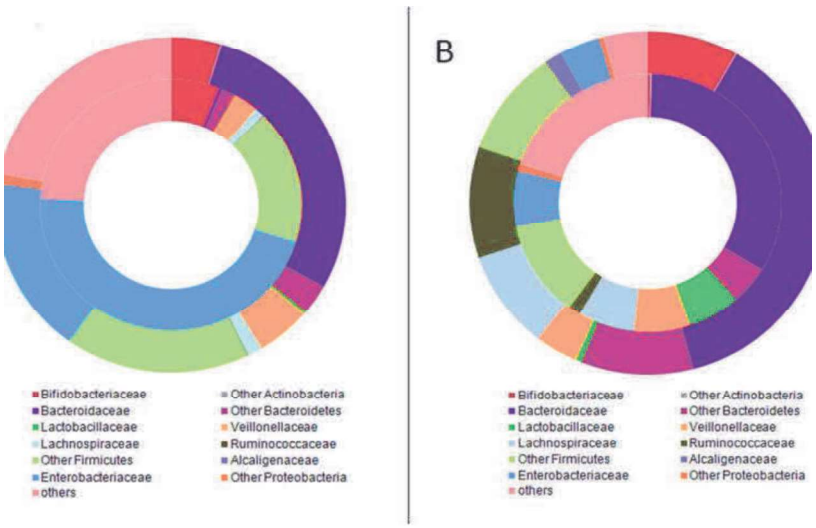
Gut microbiota in Cow's Milk Allergy (CMA)

In allergic infants, several studies show the presence of altered gut microbiota:

- Children with CMA have lower gut microbiota diversity
- Infants with IgE- mediated allergy typically have low levels of *Bifidobacteria*
- Children with non-IgE mediated allergy have dysbiosis driven by *Bacteroides and Alistipes*
- Systematic review 2023: suggest that the gut microbiome, characterized by an enrichment of the Clostridia class and reductions in the Lactobacillales order and Bifidobacterium genus, is associated with CMA in early life
- Infants who outgrew CMA were reported to have enriched Clostridia class at 3–6 months

Savo etl al. Pediatr Allergy Immunol. 2024;35:e14084. Thompson-Chagoyan OC, et al. Int Arch Allergy Immunol 2011; 156: 325-332. Kirjavainen PV, et al. Gut 2002; 51: 51–55. Soto A, et al. J Pediatr Gastroenterol Nutr. 2014 Jul; 59(1): 78–88.. Canani et al. Sci Rep. 2018 Aug 21;8(1):12500. Bunyavanich et al. J. Allergy Clin. Immunol. 2016:138;1122–1130. Petersen et al. Cell Rep. Med. 2:100260. Yang et al. Front. Microbiol. 2021.12:716667. Moriki et al. Nutrients 2022, 14, 4537

Microbial Diversity: CMA vs. No CMA



Infant microbiome and microbial diversity: no CMA vs. CMA (n=60)

Dong et al. Saudi J Biol Sci. 2018;25(5):875-80

Short chain fatty acid (SCFA) and food allergy

- Four studies found no association between three main SCFAs (butyrate, valerate, propionate) and IgE mediated food allergies. Two studies reported association with decreased level of valerate at 1y and FA at 4y and at 13y.
- Of the two studies that investigated infants with non-IgE mediated cow's milk allergy, one reported decrease in butyrate, and another reported increase in branched short-chain fatty acids.

Sasaki et al. Allergy.2024 Jul;79(7):1789-1811.

Dysbiosis contribute to infection?

The clinical burden of cow's milk allergy in early childhood: A retrospective cohort study

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Katy Sorensen[1] | Rosan Meyer[2] | Kate E. Grimshaw[3,4] | Abbie L. Cawood[1,4] | Dionisio Acosta-Mena[5] | Rebecca J. Stratton[1,4]

- A retrospective, observational study comparing clinical and healthcare outcomes among children with CMA and those without
- Data generated from the THIN database

n=6998
CMA (n=3499) vs non-CMA (n=3499)

Inclusion criteria	Exclusion criteria
- Male or female - Diagnosed with CMA and/or prescribed HAF in the last 4y 11m - Age of diagnosis and/or first prescription of a HAF ≤12m - Have received at least 3 prescriptions (3m) of HAF - Patient record flagged as 'acceptable' according to THIN	Metabolic conditions, intestinal failure, necrotizing enterocolitis, cancer/ malignancy/ tumor, cardiology, cystic fibrosis, cerebral palsy and chromosomal anomalies, patients on 'other' prescribed medical nutrition not indicated for CMA

THIN = The Health Improvement Network; HAF = Hypoallergenic formula
Sorensen et al. Immun Inflamm Dis. 2021;1–11. Open Access

Dysbiosis contribute to infection?

TABLE 4 Differences in occurrence of infections in the CMA versus non-CMA cohort

	CMA ($n = 3499$)	Non-CMA ($n = 3499$)	p-value
GI infections			
n (%)[a]	282 (8.1)	162 (4.6)	<.001
Infection rate[b]	0.105	0.065	<.001
Skin infections			
n (%)[a]	1898 (54)	1584 (45)	<.001
Infection rate[b]	1.305	0.955	<.001
Respiratory infections			
n (%)[a]	3098 (89)	2854 (82)	<.001
Infection rate[b]	6.88	5.03	<.001
Ear infections			
n (%)[a]	875 (25)	673 (19)	<.001
Infection rate[b]	0.51	0.355	<.001

- **GI infections:** viral gastroenteritis, gastroenteritis of other presumed infectious origin, campylobacter GI infection, and diarrhoea and vomiting caused by suspected infection
- **Skin infections:** skin and subcutaneous tissue infections.
- **Respiratory infections:** upper respiratory tract infection and acute tonsilitis
- **Ear infections:** otitis media, infective otitis externa, and ear pain

Sorensen et al. Immun Inflamm Dis. 2021;1–11.

Discuss the functionality and research behind B. breve M-16V.

Systematic review: The role of B. breve M-16Vand preterm health

- Meta-analysis of non-RCTs showed significant benefits on:
- late-onset sepsis-3 studies (n = 2452)
- mortality-2 studies (n = 2319),
- postnatal age at full feeds (days)-2 studies (n = 361)
- There were no adverse effects from B breve M-16V

Athalye-Jape et al. JPEN J Parenter Enteral Nutr. 2018 May;42(4):677-688.

B. breve M-16V and microbiome/immune outcomes

Patole et al. PLoS ONE 2014, 9, e89511.; Patole et al. J. Matern. Fetal Neonatal Med. 2016, 29, 3751–3755. Akiyama et al. Acta Neonatol. Jpn. 1994, 30, 130–137; Li et al. Pediatr. Int. 2004, 46, 509–515; Ishizeki et al., Anaerobe 2013, 23, 38–44.; Akiyama et al. Acta Neonatol. Jpn. 1994, 30, 257–263; Fuji et al. J. Pediatr. Gastroenterol. Nutr. 2006, 43, 83–88.; Wang et al. J. Pediatr. Gastroenterol. Nutr. 2007, 44, 252–257.

Reference	Effect of M-16V on bifidobacterial colonization in low birth weight infants
Patole et al., 2014	Significant increase in B. breve counts No probiotic sepsis and death
Patole et al., 2016	**M-16V-treated SGA infants reached full feeds earlier**
Li et al., 2004	Significant increase in bifidobacteria Significant decrease in the cell numbers of Enterobacteria
Ishizeki et al., 2013	Increase in Bifidobacterium in in Reduction in Clostridium and Enterobacteriaceae
Akiyama et al., 1994	Significant increase in bifidobacteria
Akiyama et al., 1994	Significant increase in bifidobacterial
Fuji et al., 2006	Significant increase in TGF-β1 levels
Wang et al., 2007	Significant increase in acetate to total SCFAs

B. breve M-16V and allergy outcomes

Hattori et al. *Arerugi 2003, 52, 20–30; Taniuchi et al. J. Appl. Res. Clin. Exp. Therapeut. 2005, 5, 38;* Del Giudice et al. *J. Pediatr. 2017, 43, 25;*

Reference	Type of Allergy	Study Characteristics
Hattori et al., 2003	Atopic dermatitis (eczema)	Infants aged 8.6 ± 4.5 months
		Significant increase in the proportion of Bifidobacterium Significant reduction in aerobes Significant improvement in the allergic symptoms No significant correlation between allergic symptoms and microflora.
Taniuchi et al., 2005	Food allergy	Infants aged 3.1–18.5 months with cow's milk allergy and atopic dermatitis
		Significant increase in of Bifidobacterium Significant reduction in aerobic bacteria Significant improvement in allergic symptoms
Del Giudice et al., 2017	Allergic rhinitis	Children aged 9 ± 2.2 years with pollen-induced IgE-mediated allergic rhinitis and intermittent asthma
		Significant improvement of allergic symptoms and quality of life

Explain the role of FOS & GOS as a prebiotic and its impact on digestive health.

Breast milk, human milk oligosaccharides (HMOs)

Human milk contains over 200 distinct oligosaccharides

Enhance growth of Bifidobacteria, Bacteroidetes and Lactobacillus.

Negative effect on growth of Clostridium, Enterococcus, Escherichia, Eubacterium, Lactobacillus, Staphylococcus, Streptococcus, and Veillonella spp.

Human milk contains several genera of bacteria including *Lactobacillus* and *Bifidobacterium*

Infants fed by non-secretor milk showing delay in the establishment of a bifidobacterial-laden microbiota and a more fluctuating gut microbiota

However is it important to understand that breast milk composition is very variable.

Katayama et al. Biosci Biotechnol Biochem 2016;80:621-32; Jost et al.Nutr Rev 2015;73:426-37; Garrido et al. Sci Rep 2015;5:13517; Davis et al. . Molecular & cellular proteomics : MCP 2016;15:2987-3002; Lewis et al. Microbiome 2015;3:13; Bai et al. mSystems 2018;3.

Other sources and examples of Prebiotics

- Infant milk formulae
  - A GOS/FOS mixture, and GOS alone are added to some infant milk formulas
- Asparagus, bananas, garlic, barley, chicory, leeks, onion, artichokes and tomatoes
  - Inulin is a naturally present oligosaccharide in these foods
- Breakfast cereals
  - Some cereals contain prebiotics and fibre
- Medicines
  - Lactulose is a synthetic non-metabolisable carbohydrate

Classification of dietary fibers

Category	Fiber	Fermentability by microbiome	Food sources
Non-starch polysaccharides (NSP)	Cellulose	10-30%	Grains, fruits, vegetables and nuts.
	Hemicelluloses e.g.arabinoxylan	50-70%	Cereals
	Pectins	~100%	Fruit and vegetables
	Hydrocolloids i.e. gums, mucilages, β-glucans	~100%	Gums: plant exudates, seeds and seaweed Mucilage: Natural gums Cereals: barley and oats, sorghum, rye, maize, triticale, wheat, and rice
Resistant oligosaccharides	(fructo-oligosaccharides (FOS)	100%	FOS: fruits, vegetables and cereals
	galacto- oligosaccharides (GOS)	100%	GOS: Fruit and Vegetables
	other resistant oligosaccharides)	100%	Raffinose oligosaccharides: Seeds of legumes, lentils, peas, beans, chickpeas, mallow, and mustard
Resistant starch	physically enclosed starch, some types of raw starch granules, retrograded amylose, chemically and/or physically modified starches	~100%	Whole grains, legumes, cooked and chilled pasta, potatoes and rice, and unripe bananas.
Lignin associated with the dietary fiber polysaccharides		0%	Celery and grains

Venter et al. Allergy. 2022 Nov;77(11):3185-3198

Galacto-oligosaccharides (GOS) and Frutco-oligosaccharides (FOS)

- Supplementation of infant formula with GOS/FOS mix, standard or hydrolyzed in term and preterm infants:
- Stimulate the growth of **Bifidobacteria.**
- In term infants, the bifidogenic effect of the prebiotic mixture was dose dependent and there was also a significant increase in the number of **Lactobacilli** in the supplemented group.
- Beta-2 fructans (FOS): Positive outcomes in management of diarrhea
- Preterm and term infant milk formulas, supplemented with a mixture of trans-β-galacto-oligosaccharides (TOS) and FOS as prebiotic ingredients induced a significantly higher colonization of **bifidobacteria and lactobacilli.**

Moro et al. Acta Paediatr Suppl . 2005 Oct;94(449):14-7; Lomax and Calder Br J Nutr. 2009 Mar;101(5):633-58; Miniello et al. Acta Pediatrica, 92 (441): 68-76

Prebiotics in the management of pediatric gastrointestinal disorders: Position paper of the ESPGHAN special interest group on gut microbiota and modifications

- Few studies with prebiotics in children.
- Most studies used infant formula supplemented with prebiotics
- Add-on prebiotic supplementation as a disease reduction method or management of childhood gastrointestinal disorders has rarely been reported.
- Some studies show benefits of prebiotics on selected outcomes
- We cannot give any positive recommendations for supplementing prebiotics in children with gastrointestinal disorders.

Indrio et al. Pediatr Gastroenterol Nut. 2024 Mar;78(3):728-742

Discuss the potential health benefits of prebiotics, probiotics, and synbiotics in the allergic population

Prebiotics

Role of dietary fiber in promoting immune health—An EAACI position paper

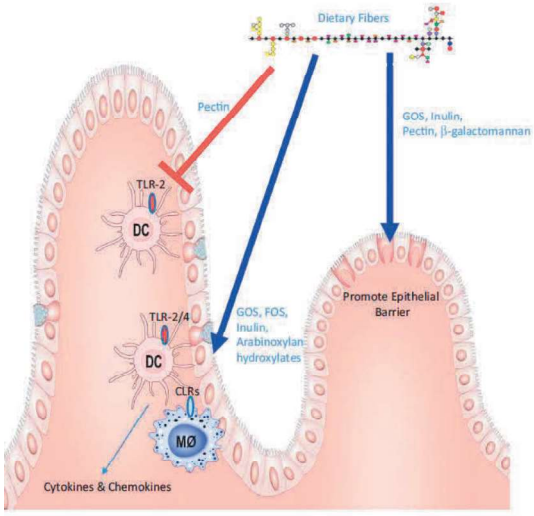


FIGURE 1 Direct effects of fibers on epithelial and immune cells. Dietary fibers such as GOS, inulins, pectins, and β-galactomannan have been shown to support a functional intestinal epithelial barrier. In addition, in vitro studies suggest that fibers including inulin, GOS, FOS, and arabinoxylan hydrolysates can modulate epithelial cell, macrophage and dendritic cell cytokine and chemokine secretion, potentially via their activation of C-type lectin receptors (CLRs) or Toll-like receptors (TLR-2 and TLR-4). In contrast, fibers such as pectin may also inhibit TLR-2-induced cytokine secretion.

Venter et al. Allergy. 2022 Nov;77(11):3185-3198.

Prebiotics and immune health

- GOS and FOS: anti-inflammatory, immune effects such as atopic disease.
  - In many instances, prebiotics **seem to be more effective when used as part of a synbiotic combination.**
- Beta2-1 fructans (FOS): Beneficial effect on immune system, reduced allergy outcomes of Respiratory Tract Infections, effect on fever and antibiotic use, of allergic disease

Marcfarlane et al. J Appl Microbiol. 2008 Feb;104(2):305-44; Lomax and Calder Br J Nutr. 2009 Mar;101(5):633-58.

HMOs and CMA – murine and human models

Murine: 2'-fucosyllactose and 6'-sialyllactose **reduced the symptoms of food allergy** through induction of IL-10+ T regulatory cells and indirect stabilization of mast cells

Human: Infants that received human milk with low Lacto-N-fucopentaose (LNFP) III concentrations (< 60µM) were more likely to become affected with cow's milk allergy when compared to high LNFP III-containing milk (odds ratio 6.7, 95% CI 2.0-22).

Castillo-Courtade et al. Allergy. 2015 Sep;70(9):1091-102.

Prebiotics and atopic dermatitis (AD)

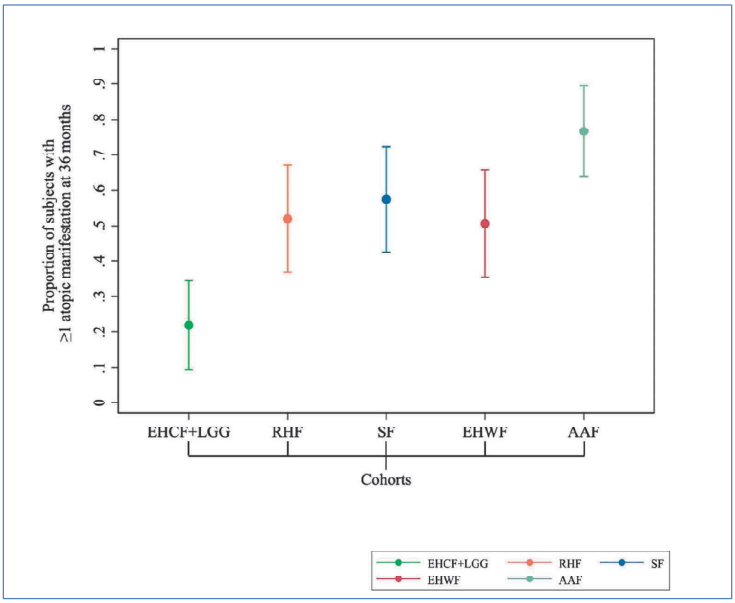
- Kestose supplementaiton in infants with AD for 12 weeks, showed a significant improvement in SCORAD scores
- Kestose is a class of sugars that belongs to a group of FOS

Shibata et al. Clin Exp Allergy. 2009;39(9):1397-1403.

Probiotics

Probiotics to manage CMA

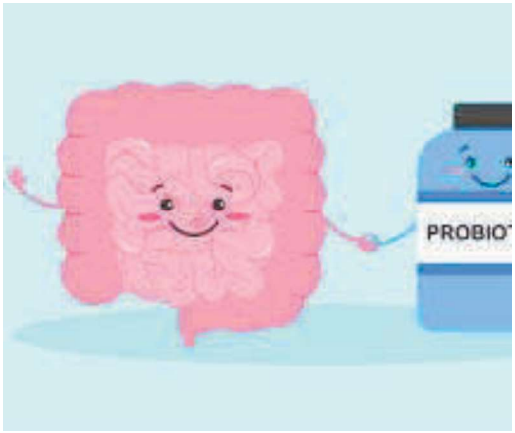
Infant diet can be manipulated **to reduce incidence of atopic manifestations** and greater rate of tolerance acquisition.



Nocerino et al. J Pediatrics 2021, Vol.232, p.183-191.e3

Probiotics and atopic dermatitis

- Although not significant, the effect size was **lower** (with 95% CI containing zero) for studies using L. rhamnosus than of those on other probiotics
- Other probiotics: Lactobacillus pentosus, Lactobacillus paracasei, Lysate from 8 common respiratory pathogenic microorganisms, Lactobacillus plantarum, Blongum, Lactobacillus casei, Lactobacillus sakei
- Effect size was lower for studies using only probiotics than for those using synbiotics.
- There was a stronger effect for prolonged administration of probiotics and ages older than 36 months.
- The dose of the supplement did not show a significant impact on the SCORAD change



Vassilopoulou et al. Allergy. 2024 Jul;79(7):1708-1724

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Very different from studies focusing on disease reduction

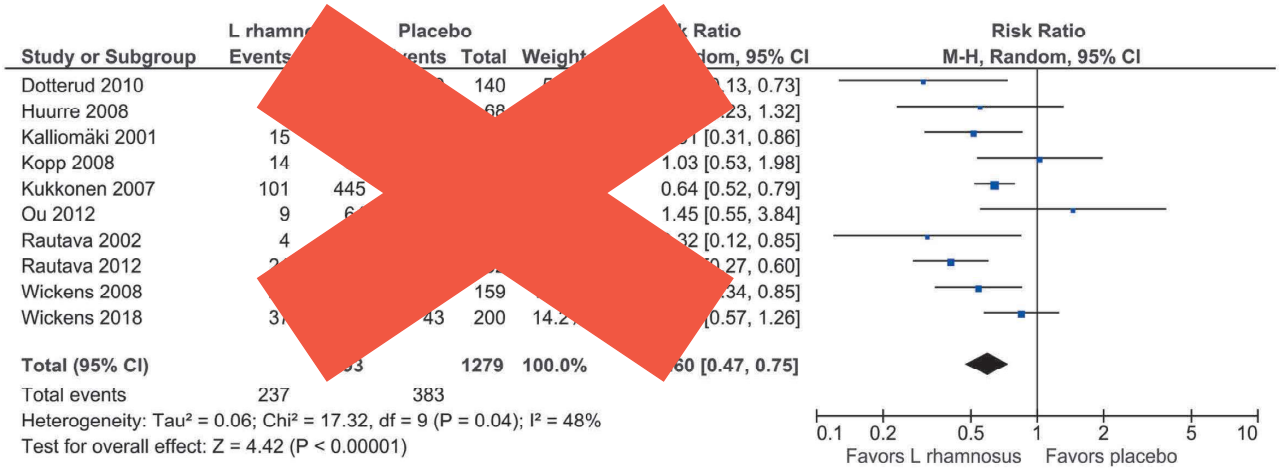

Fig. 1 Forest plot incidence of atopic eczema/dermatitis, < 2 years out

Voigt et al. Am J Clin Dermatol. 2022 Nov;23(6):801-811.; Garcia-Larsen et al. PLoS Med. 2018 Feb 28;15(2):e1002507

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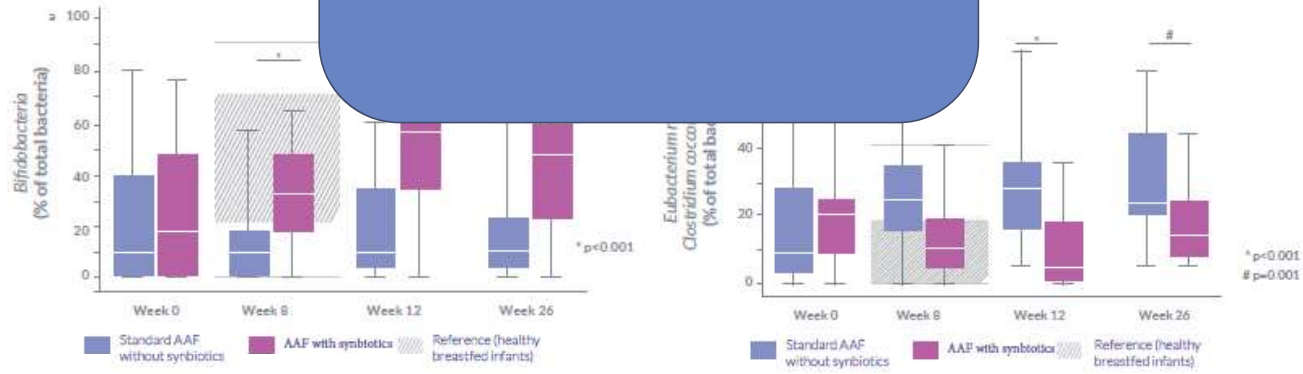
Synbiotics

Effect of Synbiotics on Dysbiosis in CMA – SEE PDF

A hypoallergenic amino ac & long chain fructo-oligosaccharides [scFOS/lc **gut microbiota closer to that of healthy b** k follow-up

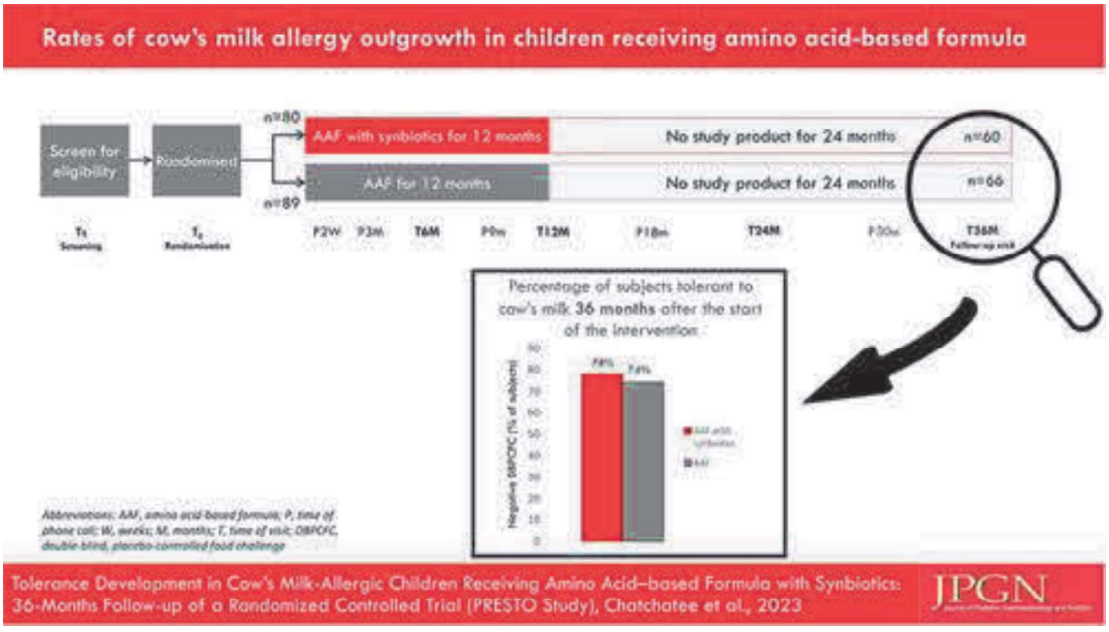
Subjects were randomized to re arides and *Bifidobacterium breve* M-16 h infants could continue study product until 26 weeks.

Use of dermatological medication and reported ear infections were lower in test versus control, p =0.019 and 0.011



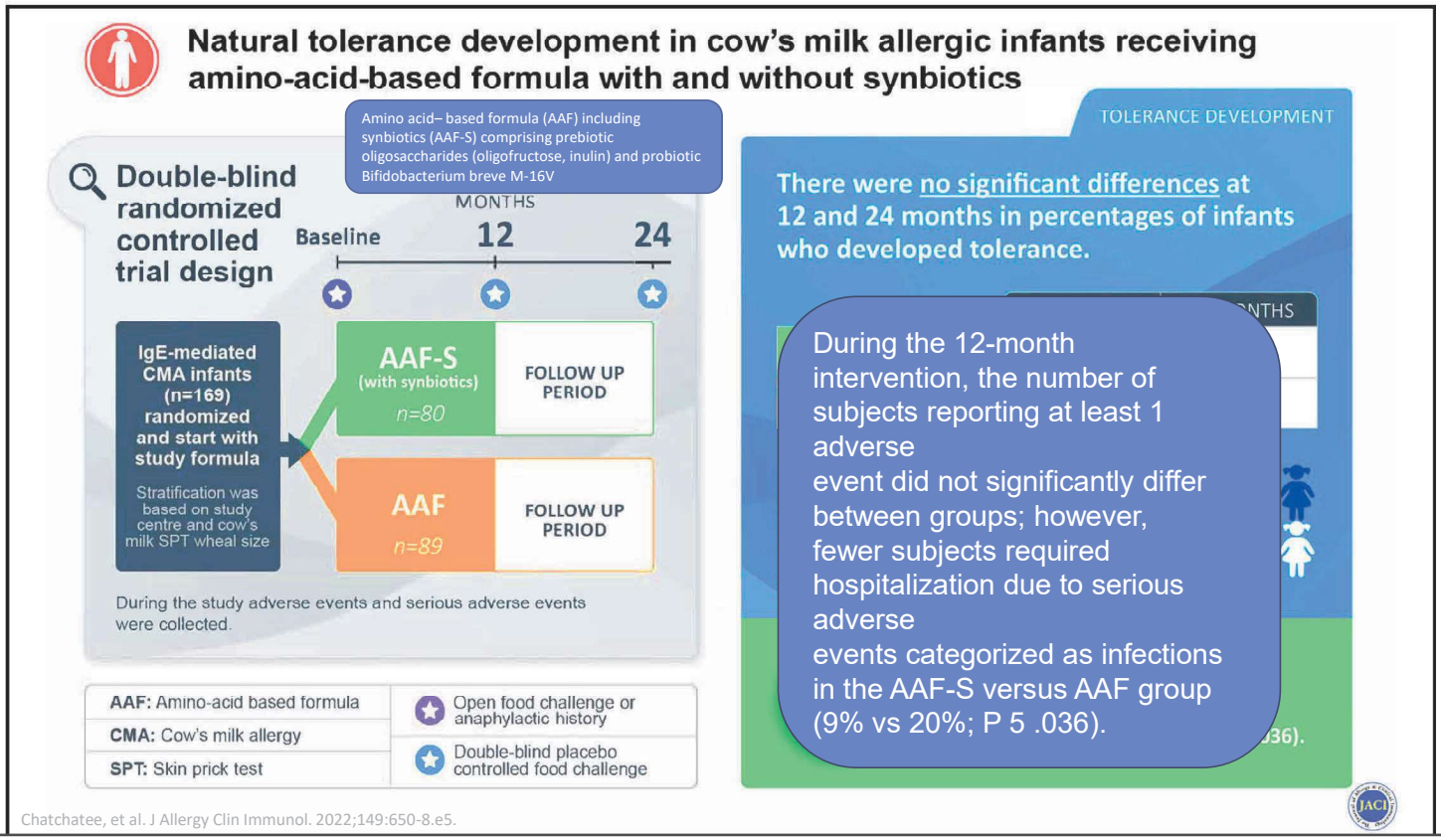
Fox et al. Clin Transl Allergy. 2019 Jan 15:9:5.

Synbiotics and cow's milk allergy: PRESTO



Chatchatee et al. J Pediatr Gastroenterol Nutr . 2024 Mar;78(3):699-703.

GRAPHICAL ABSTRACT



Chatchatee, et al. J Allergy Clin Immunol. 2022;149:650-8.e5.

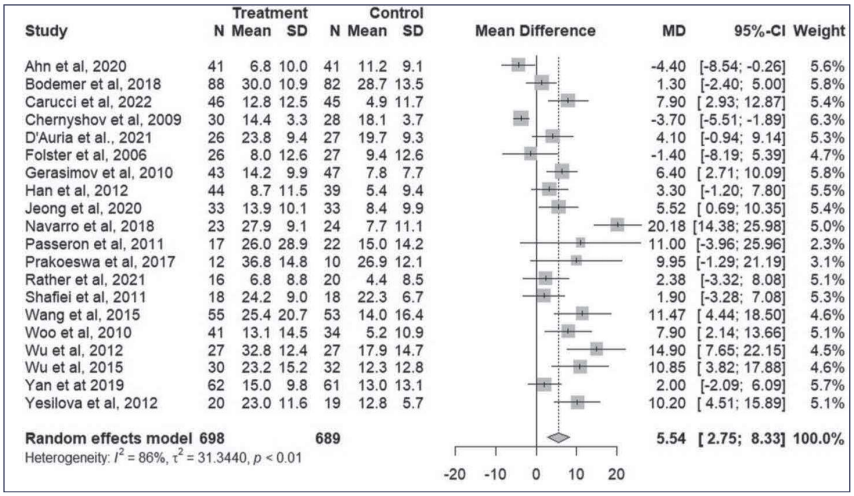
Pre, pro and synbiotics and asthma

- Prebiotics have less influence on the control of eosinophilic airway inflammation, EPO activity, immune-allergic response, and asthma.
- Short- and long-chain prebiotics (GOS and FOS), together with probiotics, may reduce allergic sensitization by regulating immune responses.
- Probiotic microorganisms can modulate immune cells such as T1, T2, T17, Treg, and B cells.
- Some studies have shown that prebiotic supplementation improves airway hyperresponsiveness and reduces the number of inflammatory cells in the sputum of asthmatics.
- Inulin (12 g/day) has also been shown to improve airway inflammation, asthma control, and gut microbiome composition

Kleniewska et al. Nutrients. 2024 Dec 24;17(1)

Probiotics/synbiotics/postbiotics and atopic dermatitis

- The average estimated effect (mean difference, MD) on SCORAD score was 5.5 (95% CI: 2.8, 8.3), meaning that a significant positive effect, favoring the management groups.
- Publication bias identified
- Significant heterogeneity
- Subgroup analysis showed no difference



Vassilopoulou et al. Allergy. 2024 Jul;79(7):1708-1724

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New (updated) guidelines and position statements/work group reports

Formula recommendations from DRACMA, GALEN AND ESPGHAN

	DRACMA	GALEN	ESGPHAN
EHF and AA based formula	Extensively hydrolyzed (milk) formula or a hydrolyzed infant rice formula can be used as the first option for managing infants with IgE and non-IgE-mediated CMA if breastfeeding is not possible or available An amino-acid formula can be a second option	The GA2LEN Task Force suggests that most infants (aged 0–1 years) diagnosed with cow's milk allergy who need a breastmilk alternative use a documented hypoallergenic extensively hydrolyzed cow's milk formula, or an amino-acid based formula if better tolerated or more appropriate.	In formula-fed infants, a CM-derived eHF is the first choice for a therapeutic elimination diet. AAF should be reserved for severe cases or infants with an absent or partial response to eHF.
Hydrolyzed rice formula	See above	The GA2LEN Task Force makes no recommendation for or against hydrolyzed plant-based formulas including rice hydrolysates that have been evaluated so far for managing food allergy in infancy.	HRFs can be considered as an alternative to CM derived eHF for therapeutic elimination diet.
Partially hydrolyzed cow's milk based formula	NA	We suggest against partially hydrolyzed cow's milk formula	Partially hydrolysed CM-based formulas (pHF) are not recommended in the management of CMA
Soy formula	A soy infant formula would be regarded as the last option	We suggest against soy-based formula in infants under 6 months	Soy protein-based formula is not recommended for infants <6 months, but may be used in the management of CMA in infants because of economic and cultural reasons (and better palatability).
Pre/pro/synbiotics	Formulas without a probiotic or an extensively hydrolyzed (milk) formula containing Lacticaseibacillus rhamnosus (formerly Lactobacillus rhamnosus) GG can be used for infants with either IgE or non-IgE-mediated CMA.	The GA2LEN Task Force makes no recommendation for or against any prebiotics, probiotics or synbiotics that have been evaluated so far for managing food allergy, whether used as a supplement or added to infant formula.	There is insufficient evidence demonstrating that the addition to eHFs of pro-, pre- or synbiotics studied so far improves their therapeutic efficacy.

Muraro, et al.. World Allergy Organiza. 2022;15(9):100687.; Venter et al. World Allergy Organ J. 2024; https://www.worldallergyorganizationjournal.org/article/S1939-4551(24)00062-0/fulltext; Vandenplas et al.Pediatr Gastroenterol Nutr. 2023.

In summary

- Allergic diseases are characterized by changes in the gut microbiome and dysbiosis
- Pre, pro and synbiotic intake leads to changes in the gut microbiome and may reduce infections
- The role of these substances in the management of allergic diseases seems promising
- The role of these substances in tolerance induction, particularly in cow's milk allergy remains to be seen

Thank you – any questions?

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