The Benefits of

Lactose & Human Milk Oligosaccharide (HMO)

in Infant Nutrition

Mohammad Gharagozlou MD

Allergy & Clinical Immunology Dept.

Children's Medical Center

Tehran University of Medical Sciences

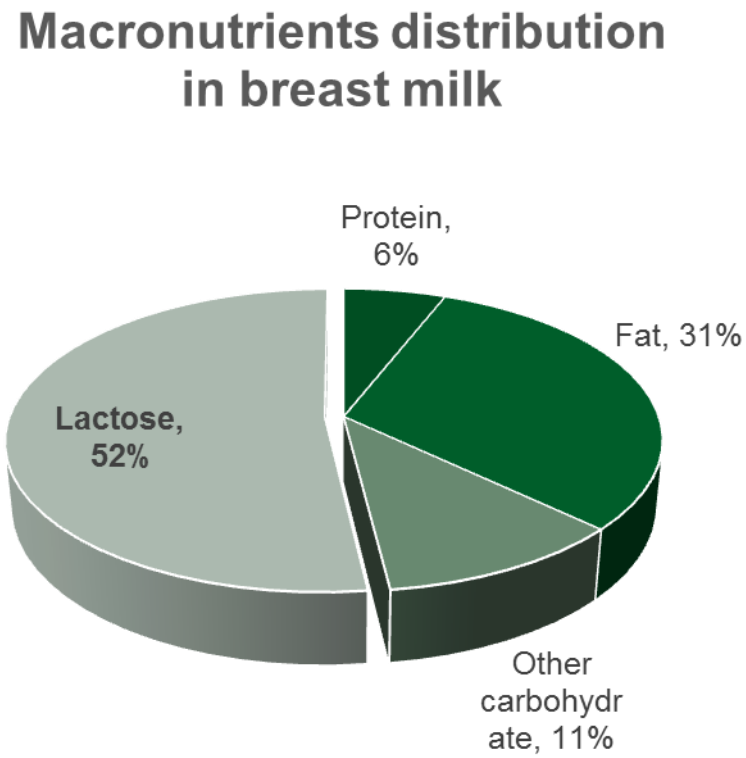
19.5.2022

LEARNING OBJECTIVES

- What are lactose and HMO?
- Lactose intolerance(LI), clinical manifestations and differences with CMPA
- The role of LF formula
- The categories of HMO found in human milk
- The most abundant HMO in human milk
- The structure of HMO and difference between them and other oligosaccharides
- Factors influencing the composition of HMO
- Human milk versus other mammalian milks – Why it is the gold standard?

LACTOSE (β-galactosyl-1,4 glucose

- Main carbohydrate in human and mammalian milk
- Human milk contains 7.5 g/100 ml of lactose, compared to about 5 g/100 ml in cow's and other mammalian milk
- Young infants do not absorb all of the ingested lactose from breast milk (physiological lactose malabsorption)
- A term infant is able to digest about 60-70 g of lactose /day (= 1 litre of breast milk)



2. Wojcik KY et al. 2009
3. Bissett DL et al. 1974
4. Bode L 2012

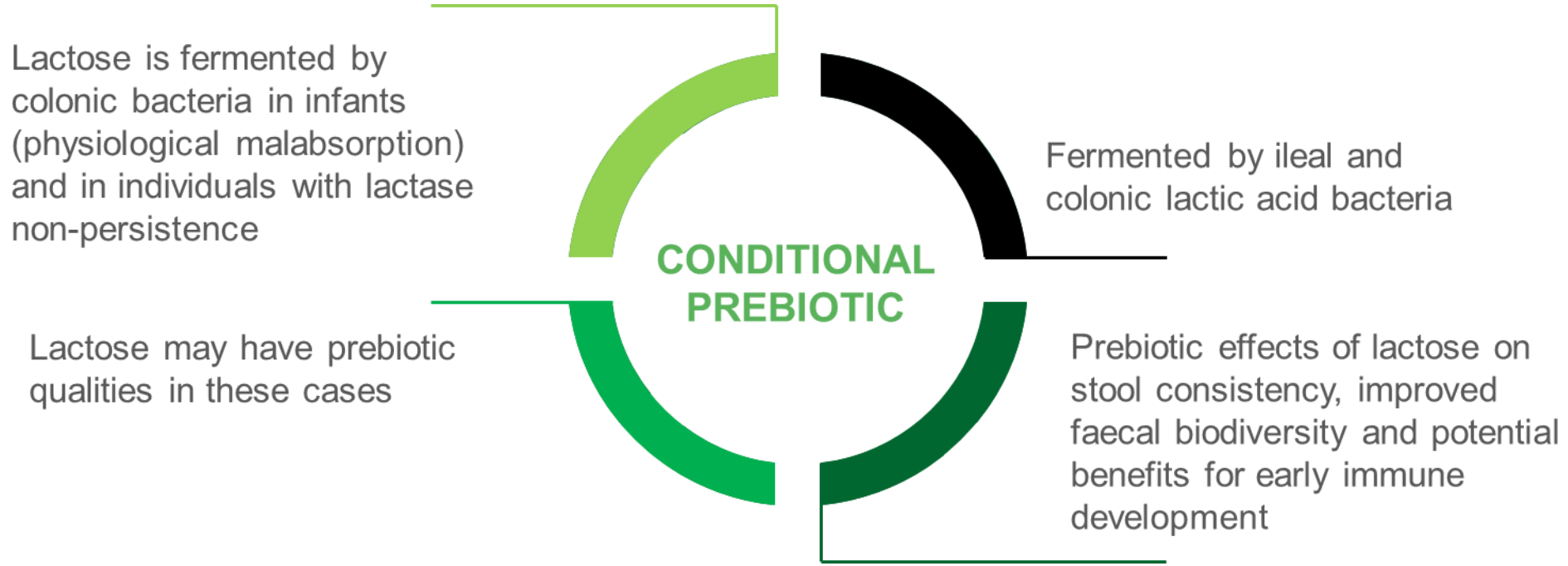
LACTASE-PHLORIZIN HYDROLASE

Commonly called lactase, hydrolyses lactose into D-glucose and D-galactose

- Small intestinal brush border enzyme (160 kDa)
- Lactase gene located on chromosome 2
- Member of b-galactosidase family
- Expressed by mature enterocytes (highest expression in mid-jejunum)
- Also hydrolyses phlorizin and some dietary glycolipids
- Peak lactase activity in term infants

1. 5. Dahlqvist A 1961
2. 6. Dahlqvist A et al. 19638. Norén O et al. 2001
3. 9. Antonowicz I & Lebenthal E 1977
4. 10. Buller HA et al. 1989
5. 11. Herzler SR and Saviano DA 1996
6. 7. Auricchio S et al. 1965

LACTOSE AS A ‘CONDITIONAL PREBIOTIC’



Francavilla et al. 2012
Szilagyi A 2004
West et al. 2015

CLINICAL CLASSIFICATION OF LI

The four main clinical types:

- Developmental lactase deficiency
- Congenital lactase deficiency - alactasia
- Lactase non-persistence (LNP) - hypolactasia
- Secondary lactase deficiency

Heine RG et al. 2017

CAUSES & MECHANISM OF SECONDARY LACTASE DEFICIENCY IN INFANTS

Diagnosis	Mechanism
Gastroenteritis	Transient (postenteritic malabsorption)
Cow's milk enteropathy	Transient (villous damage)
Celiac disease	Transient (villous damage)

CLINICAL PRESENTATION OF LI

Symptoms generally occur within 30-60 min of ingesting lactose containing foods.
Differs between infants and older children

INFANTS

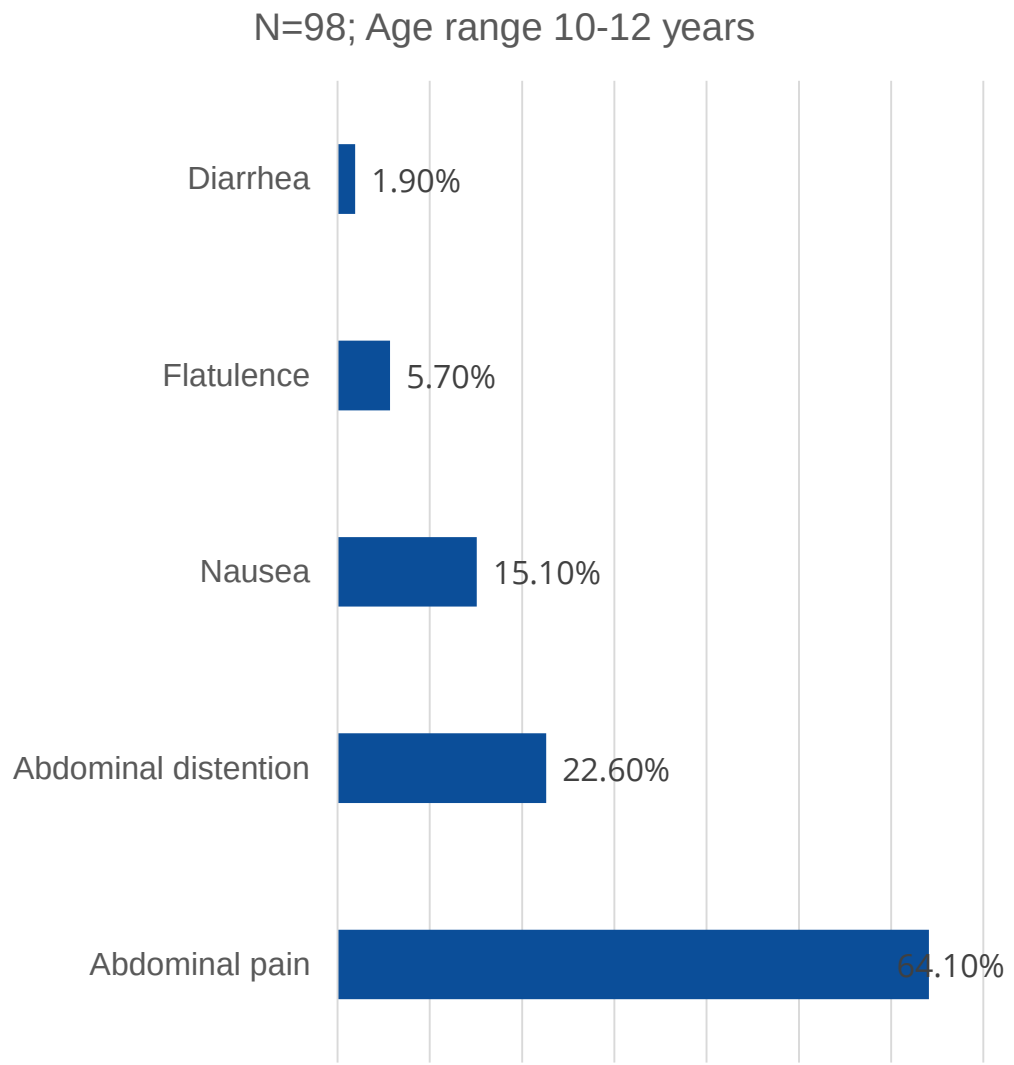
- More prone to develop diarrhea

(lacks the ability to compensate by colonic reabsorption)

- A low fecal pH (<5.5) may cause perianal skin irritation and excoriation

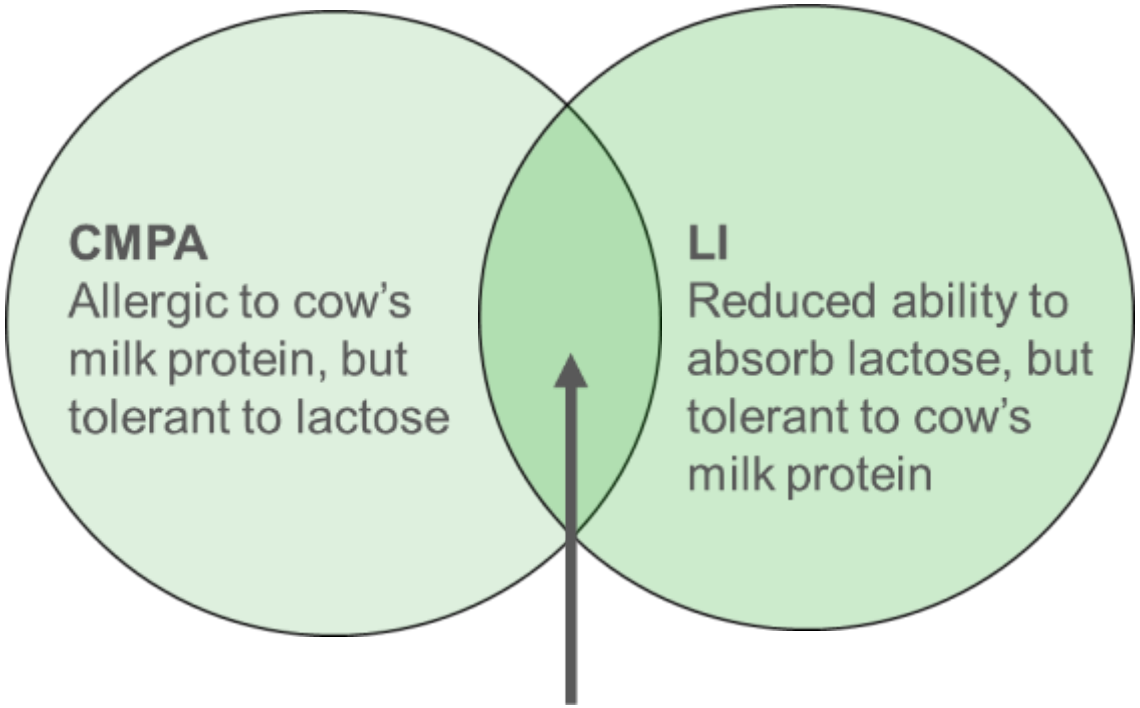
OLDER CHILDREN & ADULTS

- Re-absorption of fermentation products reduces the osmotic load and significantly reduces diarrhea



Heine RG et al. 2017
Hegar B & Widodo A, 2015

CLINICAL OVERLAP BETWEEN CMPA AND LI

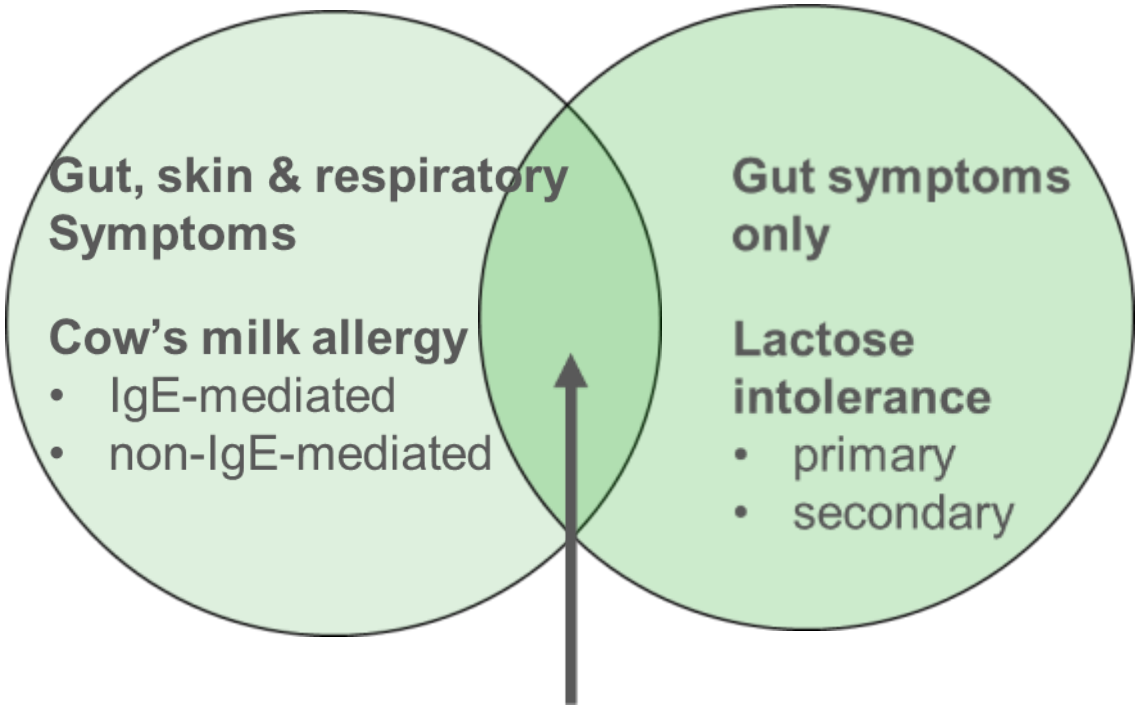


Cow's milk enteropathy with secondary lactase deficiency

Allergic to cow's milk protein and reduced ability to absorb lactose

1. Heine RG et al. 2017

CMPA & LI – TWO DIFFERENT CONDITIONS



Cow's milk enteropathy
with secondary LI
- non-IgE-mediated

PERSISTENT DIARRHEA POOR GROWTH IRON DEFICIENCY ANEMIA

Heine RG et al. 2017

TYPICAL SYMPTOMS OF GASTROINTESTINAL CMPA AND LI

	Gastrointestinal CMPA	LI
Diarrhoea	+++	+++
Vomiting	++	-
Rectal bleeding	+	-
Perianal rash	++	+++
Eczema	+	-
Poor weight gain	+++	++

Heine RG et al. 2017

GENERAL TREATMENT PRINCIPLES

- Continue breastfeeding in breastfed infants
- In formula-fed infants, a limited trial of lactose-free formula may be indicated (e.g. following viral gastroenteritis)
- Reduce (do not eliminate completely) the intake of lactose-containing foods
- Small amount of lactose in divided doses usually tolerated
- In older children and adolescents, up to 12-24g of lactose often tolerated, if taken in small doses divided through the day.

1. Heine RG et al. 2017

CAUSES, MECHANISM AND TREATMENTS OF SECONDARY LACTOSE INTOLERANCE

Diagnosis	Mechanism	Treatment
Viral Gastroenteritis	Transient (postenteritic malabsorption)	Short-term lactose restriction
Cow's milk enteropathy	Transient (villous damage)	Cow's milk protein-free diet (until tolerant to CMP)
Celiac disease	Transient (villous damage)	Life-long gluten and short-term lactose restriction

Heine RG et al. 2017

MISDIAGNOSIS OF LI AND CMPA

- Persistent symptoms due to cow's milk enteropathy often not recognized
- HCP often unaware of clinical differences and confused about different infant formula types

  Inappropriate use of lactose-free or lactose-reduced formula in infants with CMPA
- Transient nature of secondary lactose intolerance often not recognised

Heine RG et al. 2017
Wauters L et al. 2016

LACTOSE RESTRICTION RARELY NEEDED IN INFANTS WITH CMPA

Type of CMPA	Need for lactose restriction
IgE-mediated CMPA/anaphylaxis	NO
Cow's milk protein-induced enteropathy	YES *
Cow's milk protein-induced enterocolitis syndrome (FPIES)	NO
Cow's milk protein-induced proctocolitis	NO
CMPA-associated gastro-esophageal reflux disease	NO
CMPA-associated constipation	NO
CMPA-associated eczema	NO

* until intestinal mucosal repair has occurred on CM-free diet

Heine RG et al. 2017

THE ROLE OF LACTOSE IN EHF

- eHF is the first choice of formula in most infants with CMPA that are not breastfed
- A lactose-containing formula is well tolerated by most infants with CMPA
- An eHF without lactose is only warranted if an enteropathy with secondary lactose intolerance is present
- Lactose may cautiously be introduced after about 1-2 months, once symptoms have resolved and small intestinal lactase activity has been restored

Heine RG et al. 2017
Koletzko S et al. 2012

THE ROLE OF LACTOSE IN EHF- GUT MICROBIOTA

Lactose in eHF has several beneficial effects on the

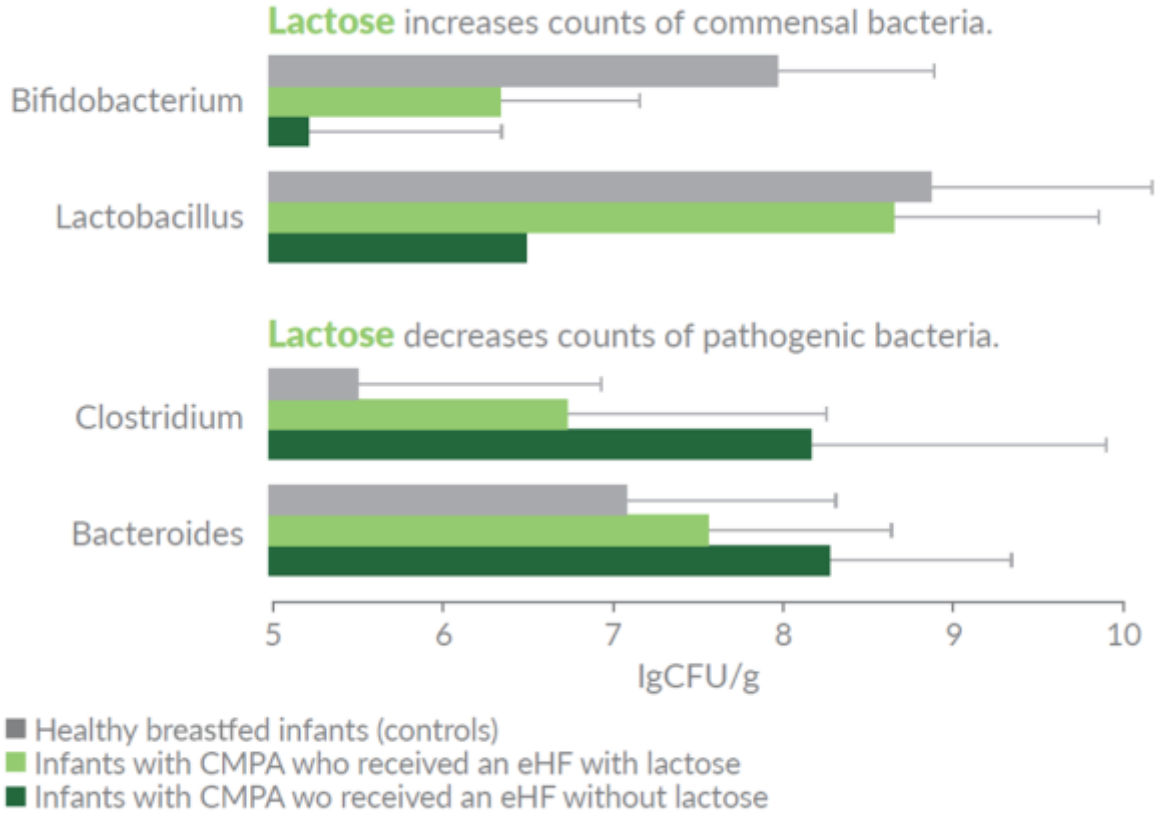
- gut microbiota with significant:
  - increase in Lactobacilli & Bifidobacteria
  - decrease in Bacteroides & Clostridia
- fecal metabolome with increased concentrations of short chain fatty acids (SCFA)
  - acetic and butyric acid

- These prebiotic effects of lactose are thought to have positive effects on early immune development

Francavilla et al. 2012
Szilagyi et al. 2010
West C et al. 2015

Microbiota in fecal samples from infants with CMPA who received an eHF with or without lactose

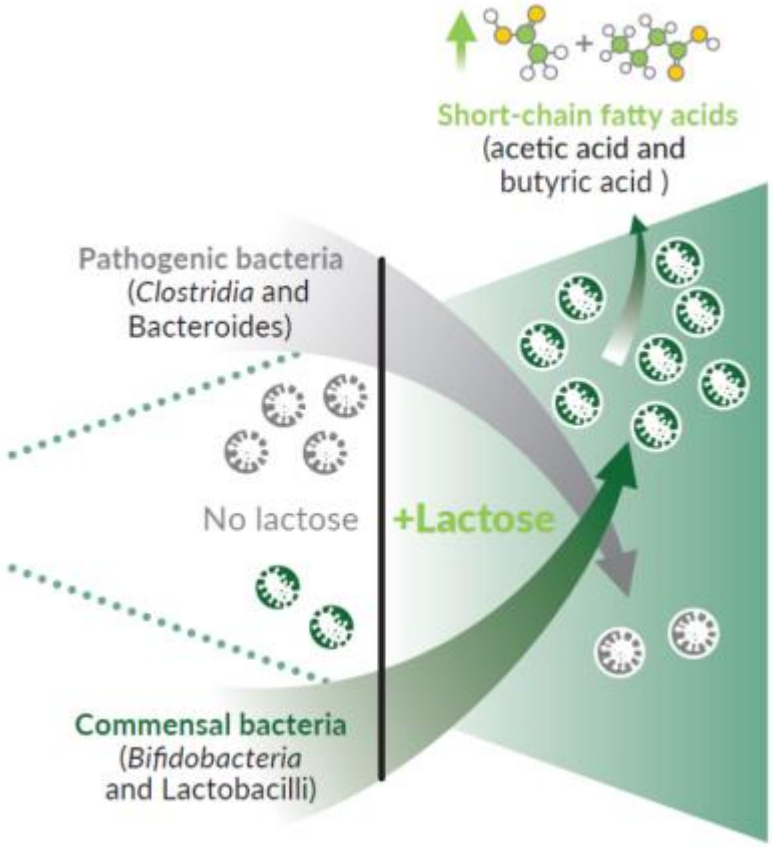
- Significant increase in bifidobacteria and lactic acid bacteria ($p<0.05$) **reaching counts found in healthy controls**
- Significant increase in Bacteroides/clostridia ($p<0.05$)



Francavilla et al. 2012

SCFA in fecal samples from infants with CMPA who received an eHF with or without lactose

- Significant increase in concentrations of total SCFA ($p<0.03$)
- Major differences found for acetic acid and butyric acid.
- Potential for lactose to promote SCFA-producing bacteria



Francavilla et al. 2012

THE ROLE OF LACTOSE IN EHF - CALCIUM ABSORPTION

Lactose in infant formula significantly increases the absorption of calcium

Calcium (n=18)	Lactose-containing formula	Lactose-free formula
Intake (mg/d)	507 ± 105	500 ± 91
Percentage absorption (%)	66.5 ± 11.9	56.2 ± 15.3[2]
Total absorption (mg/d)	339 ± 88	279 ± 85[3]

[1] mean ± SD

[2, 3] Significantly different from lactose-containing formula (paired *t* test):
[2]*P* = 0.002, [3]*P = 0.006.*

Abrams SA et al. 2002

THE ROLE OF LACTOSE IN EHF- TASTE

- Most hypoallergenic formulas are lactose-free and many are poor tasting

- The addition of lactose slightly increases the sweetness of EHF
  - Improves the overall palatability
  - Reduced risk of taste aversion and formula refusal, particularly in older infants

Miraglia Del Giudice et al. 2015

THE ROLE OF LACTOSE IN EHF - CLINICAL & IMMUNOLOGICAL BENEFITS

'Given the positive effects of lactose on fecal microbiome and metabolome, lactose-containing eHF may offer clinical and immunological benefits in the treatment of infants with CMPA'

1. Heine RG et al 2017

HMO – a complex mix of carbohydrates and bioactive compounds "*nursing the microbiota*"[4]

A complex mix of (predominantly) non-digestible carbohydrates[1-3]

- ~1% to 2% are absorbed
- ~98% to 99% metabolised by microbes or excreted

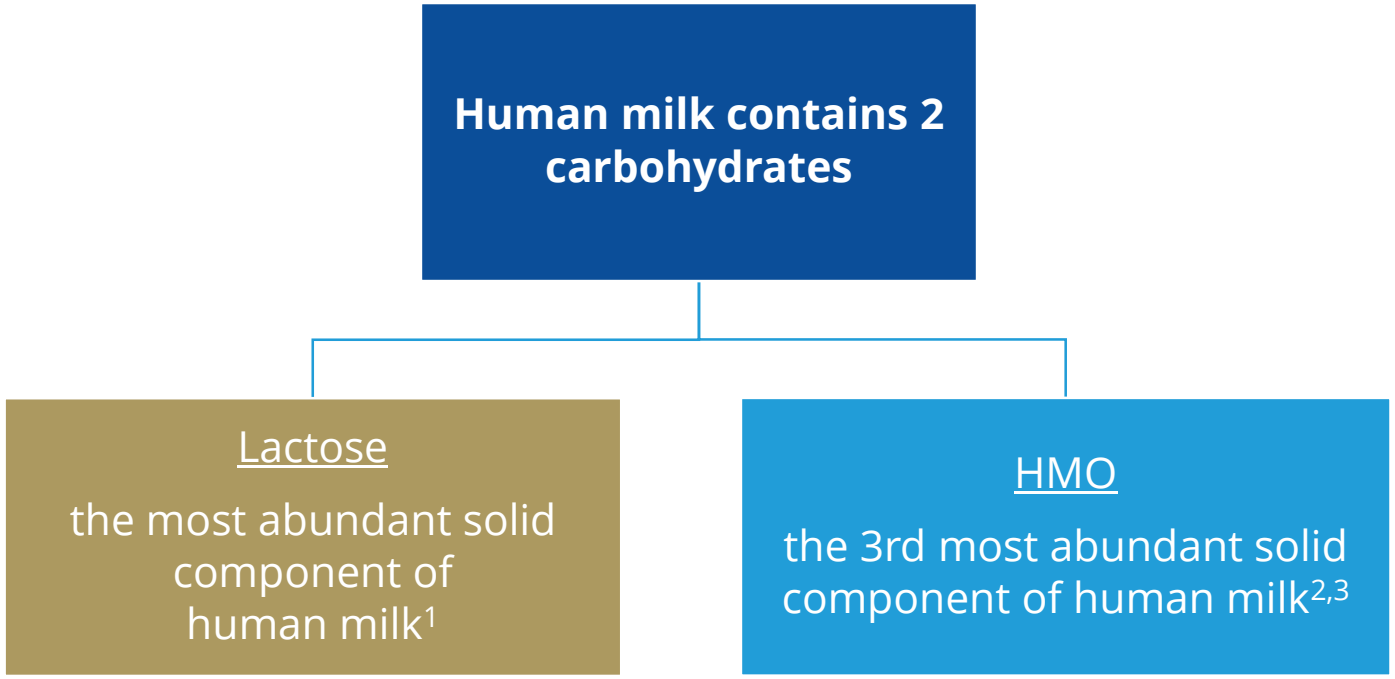
HMO act as bioactive compounds – present in high amounts in human milk[4]

HMO support the infant gut microbiota[5]

HMO, human milk oligosaccharide.

1. Ruhaak LR, et al. *Anal Bioanal Chem*. 2014.
2. Rudloff S, et al. *Br J Nutr*. 2012.
3. Rudloff S. & Kunz C. *Adv Nutr.* 2012.
4. Donovan S, Comstock S. *Ann Nutr Metab.* 2016.
5. Sela D, Mills D. *Trends Microbiol.* 2010.

Carbohydrates in human milk



The amount and variety of HMO is unique to each individual mother[3-5]

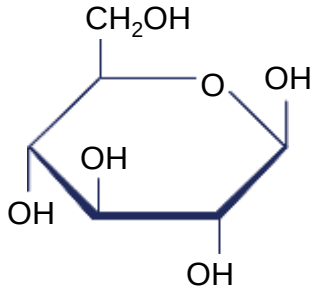
HMO, human milk oligosaccharide.

1. Pacheco AR, et al. *Annu Rev Anim Biosci.* 2015.
2. Plaza-Díaz J, et al. *Nutrients.* 2018.
3. Ruhaak LR, et al. *Anal Bioanal Chem*. 2014.
4. Samuel TM, et al. *Sci Rep.* 2019.
5. Tonon KM, et al. *Nutrients.* 2019.

HMO – "*Every baby needs a sugar mama*"[1]

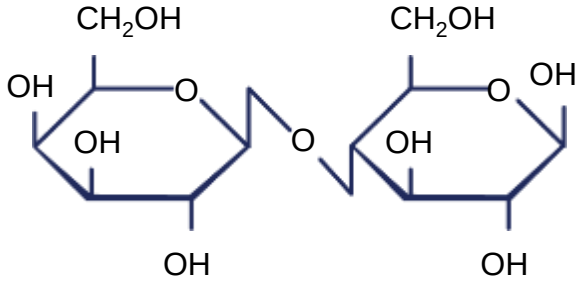
- Carbohydrates also called sugars/saccharides can be classified as:[2]

Monosaccharides
e.g. glucose
(1 monosaccharide molecule)



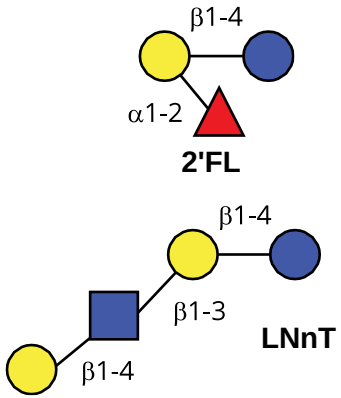
Glucose
$C_6H_{12}O_6$

Disaccharides
e.g. lactose
(2 monosaccharide molecules linked)

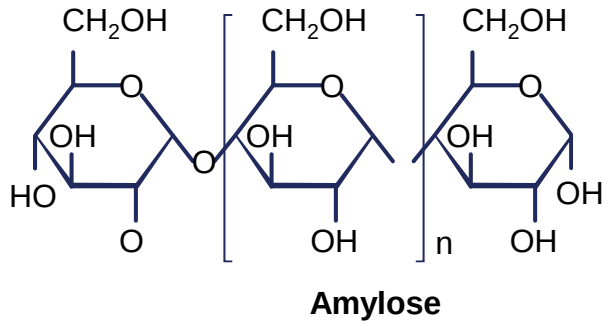


Lactose
$C_{12}H_{22}O_{11}$

Oligosaccharides
e.g. HMO (3-10 monosaccharide molecules linked)



Polysaccharides
e.g. starch
(>10 monosaccharide molecules linked)



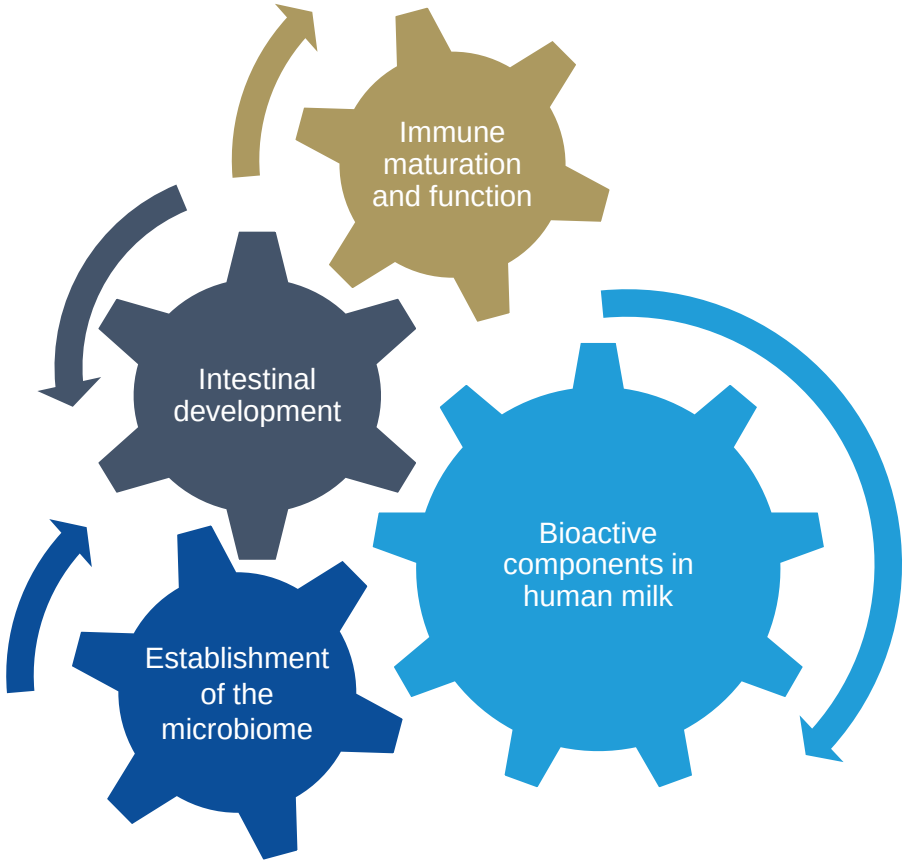
Amylose

The building blocks of HMO are: Glucose + galactose (lactose) plus one or more of N-acetyglucosamine, fucose and N-acetylneuraminic acid (sialic acid)[1]

HMO, human milk oligosaccharide.

1. Bode L. *Glycobiology.* 2012.
2. Lunn J, Buttriss JL. *Nutrition Bulletin.* 2007.

HMO as bioactive compounds – impacting the overall health of the individual



Human milk contains a variety of factors and qualities that have a profound role to play in infant survival and health[1]

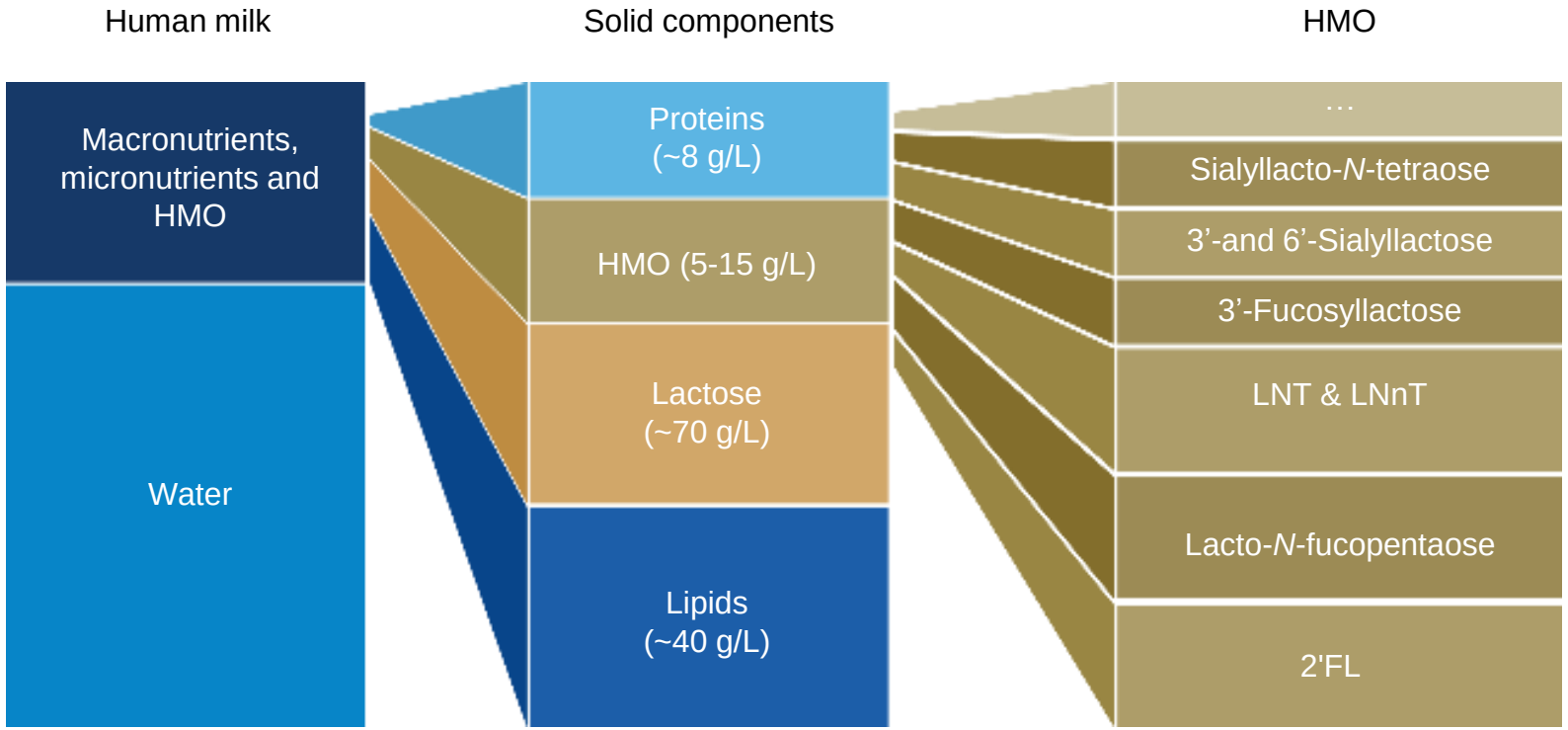
Among the bioactive components in human milk that are modulators of these processes, oligosaccharides are a key component[2,3]

HMO affect biological processes or substrates and have an impact on body function/condition - and ultimately health[1]

HMO, human milk oligosaccharide.

1. Ballard O, Morrow A. *Pediatr Clin North Am.* 2013.
2. Donovan S. & Comstock S. *Ann Nutr Metab.* 2016.
3. Pfeuffer M. & Schrezenmeir J. *Br J Nutr*. 2000.

HMO are the 3rd largest solid component in human milk



Adapted from Zivkovic, et al. *PNAS.* 2013.

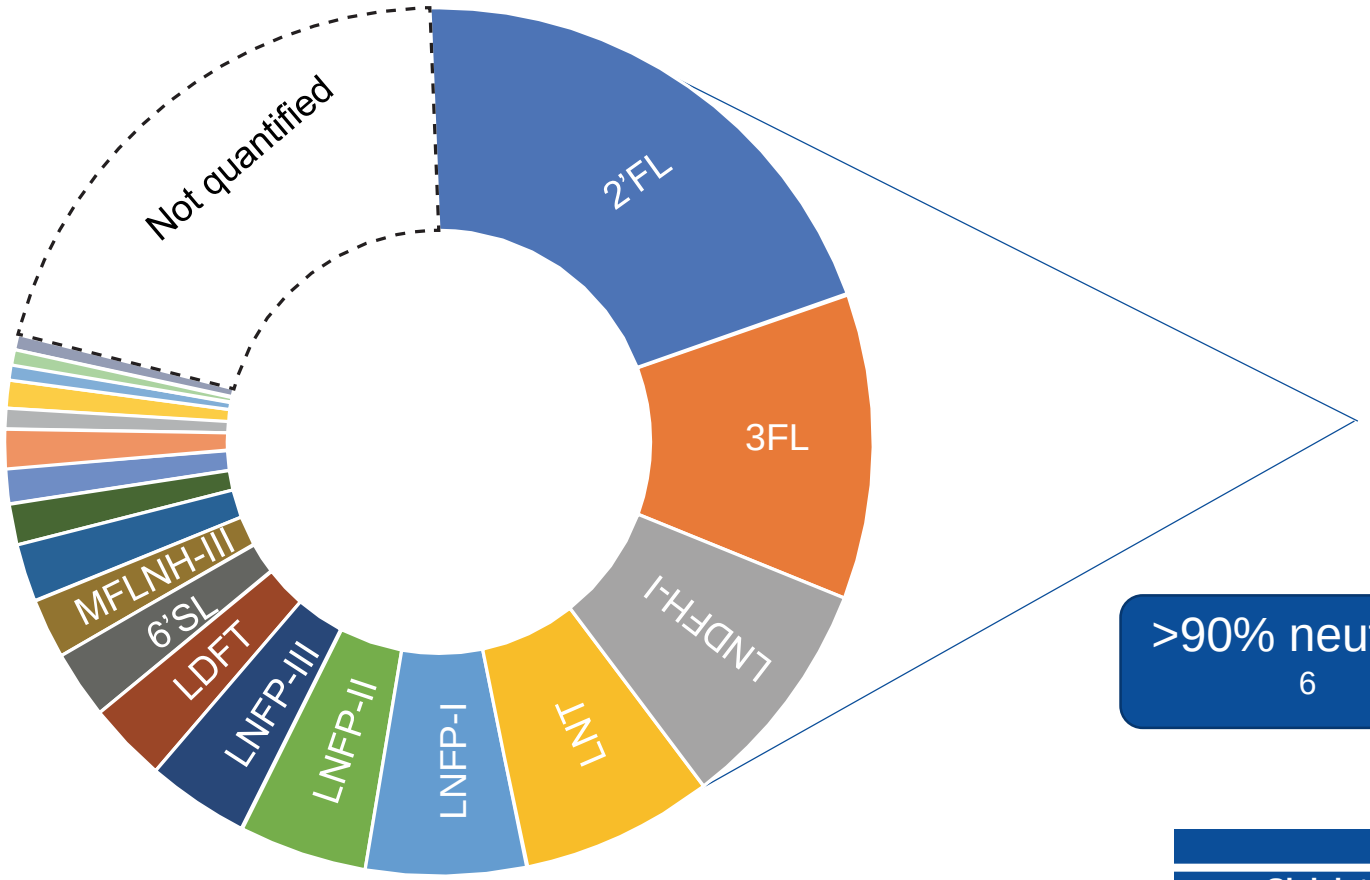
The HMO fraction is quantitatively larger than that of protein (which is typically around 8 g/L)[1,2]

HMO can therefore be considered a key component of breast milk[1]

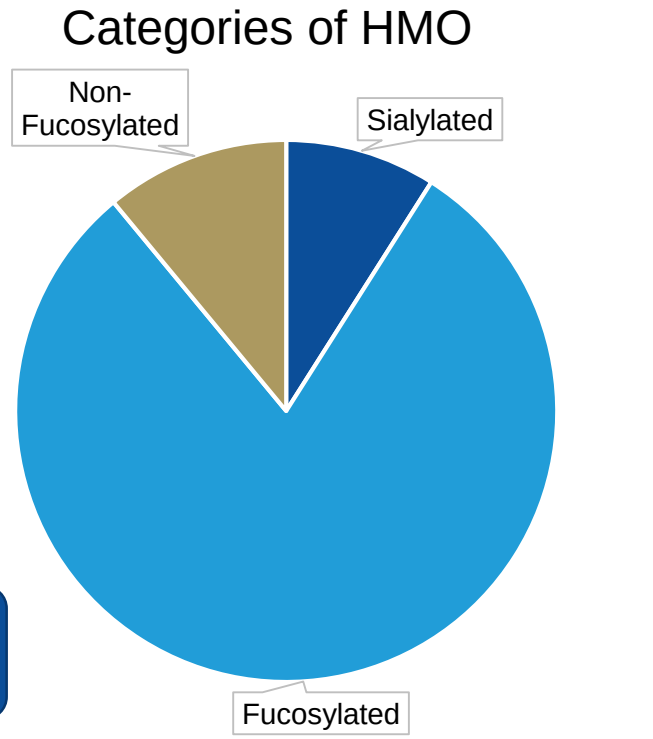
2'FL, 2'fucosyllactose; HMO, human milk oligosaccharide; LNnT, lacto-N-(Neo)tetraose.

1. Zivkovic A, et al. *PNAS.* 2011.
2. Coppa GV, et al. *Acta Paediatr.* 1999.
3. Zivkovic A, et al. *Funct Food Rev*. 2013.

Around 200 HMO have been identified and classified[1]



>90% neutral[2-6]



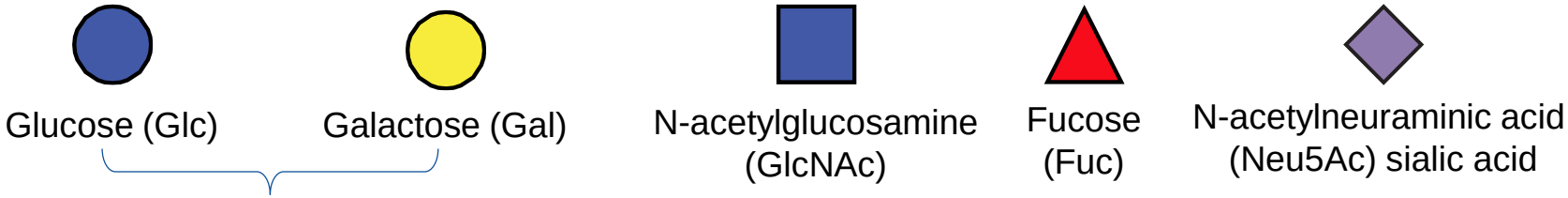
	mg/L	%
Sialylated (acidic)	669	9%
Fucosylated (neutral)	5720	80%
Non-Fucosylated (neutral)	801	11%

1. Ninonuevo MR, et al. *J Agric Food Chem*. 2006.
2. Austin S, et al. *Nutrients.* 2016.
3. Austin S, et al. *Nutrients.* 2019.
4. Sprenger N, et al. *PLoS ONE.* 2017.
5. Samuel TM, et al. *Sci Rep*. 2019.
6. Lefebvre et al. 2020 (manuscript submitted).

2'FL, 2'fucosyllactose; 3'FL, 3'fucosyllactose; 3'SL, 3'sialyllactose; 6'SL, 6'sialyllactose; HMO, human milk oligosaccharides; LDFT, lactodifucotetraose; LNDFH-I, lacto-N-difucohexaose; LNFP, lacto-N-fucopentaose; LNT, lacto-N-tetraose; LNnT, lacto-N-neotetraose; MFNLH-III, monofucosyllacto-N-hexaose-III.

Structure of HMO

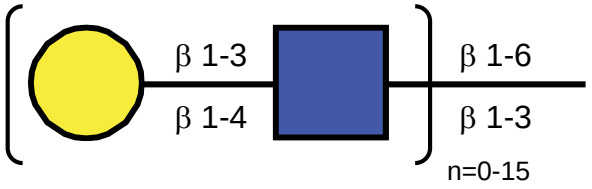
HMO are composed of 5 monosaccharide units[1]



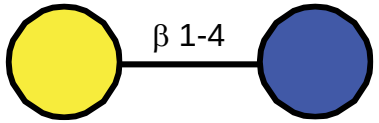
General structure that applies to all HMO[1-3]

Terminal positions
N-acetylneuraminic acid
L-Fucose

Core structures
Lacto-*N*-biose (β1-3)
N-acetyllactosamine (β1-4)



Lactose core
D-Lactose



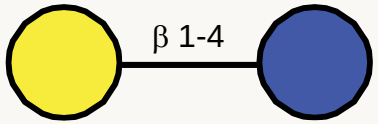
HMO, human milk oligosaccharide.

1. Zivkovic A, et al. *Proc Natl Acad Sci.* 2011.
2. Bode L. & Jantscher-Krenn E. *Adv Nutr.* 2012.
3. Jantscher-Krenn E. & Bode L. *Minerva Pediatr.* 2012.

HMO are structurally diverse with a lactose backbone

HMO commonly have a lactose backbone or core[1]

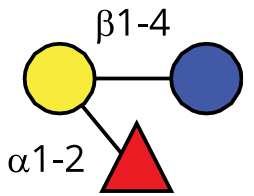
Lactose core
D-Lactose



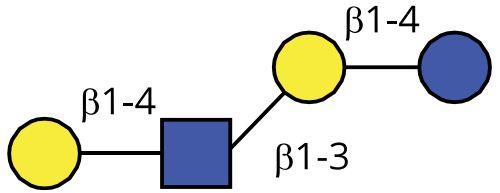
Components:

- Glucose
- Galactose
- N-acetylglucosamine
- Fucose
- Sialic acid

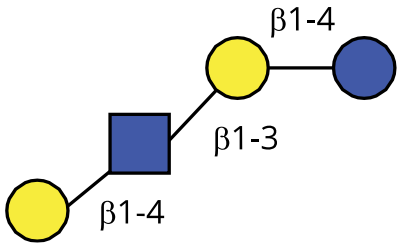
2'FL



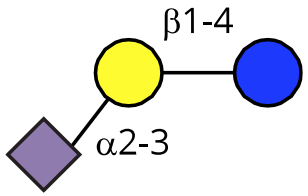
LNnT



LNT



3'-SL



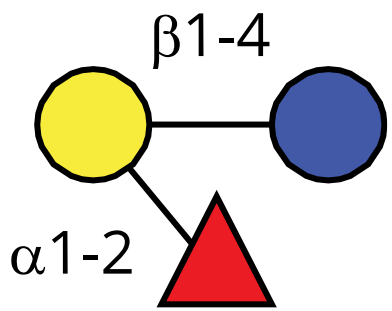
2'FL, 2'fucosyllactose; HMO, human milk oligosaccharide; LNnT, lacto-N-neotetraose; LNT, lacto-N-tetraose; 3'-SL, 3'-sialyllactose.

1. Bode L. *Early Hum Dev.* 2015.

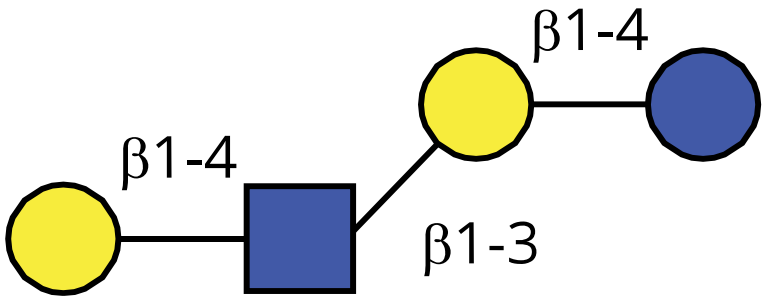
2'FL and LNnT are among the most abundant HMO in human milk

2'FL and LNnT are neutral oligosaccharides[1]

2'FL



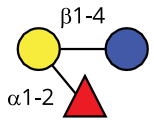
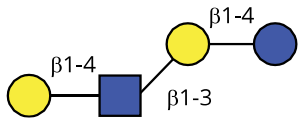
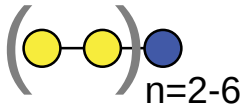
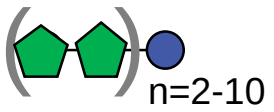
LNnT



2'FL, 2'fucosyllactose; HMO, human milk oligosaccharide; LNnT, lacto-N-neotetraose.

1. Erney R, et al. *JPGN.* 2000.
2. Vandenplas Y, et al. *Nutrients.* 2018.

HMO have a unique structure different from GOS and FOS

	HMO	GOS[5,6]	FOS
Found in breast milk	**+++**	**+/-**	**-**
Structure[1-3]	2'FL  LNnT 		

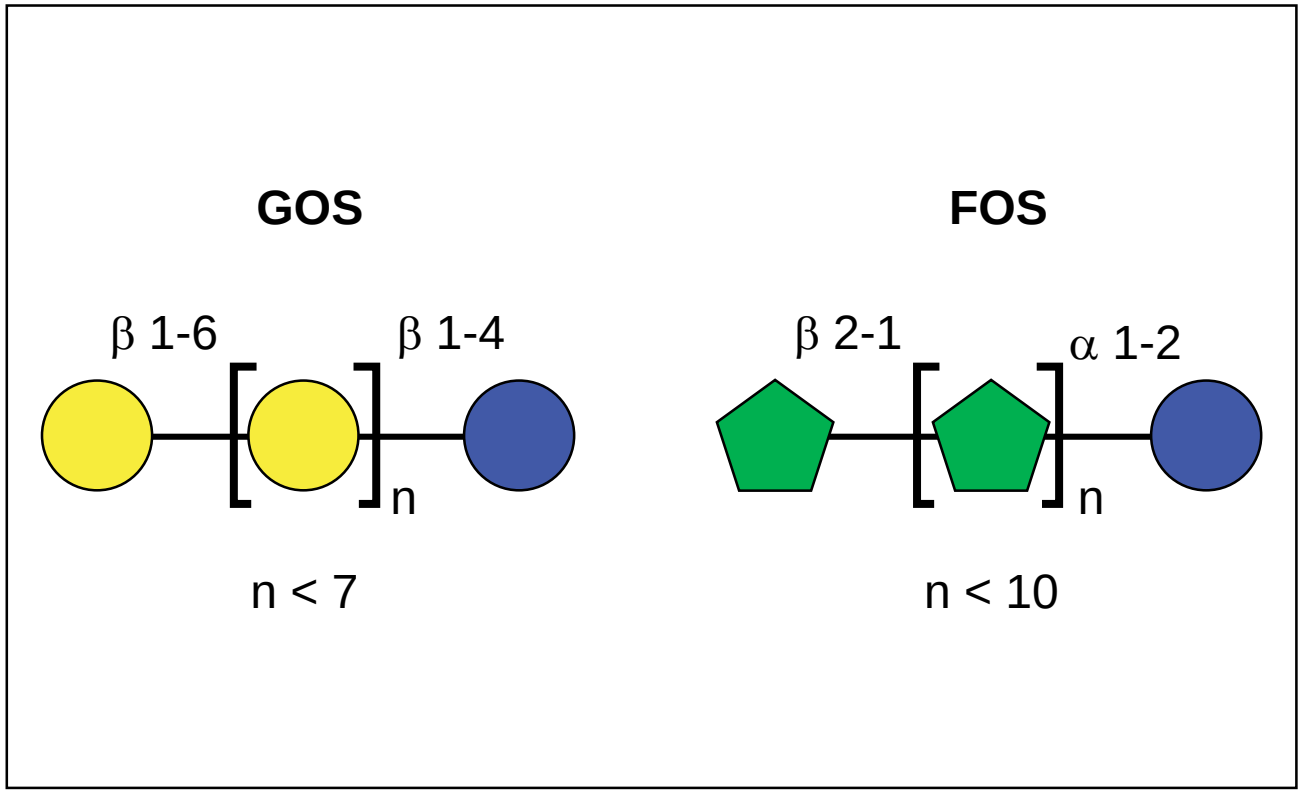
Glucose Galactose N-acetylglucosamine-galactose Fucose Fructose

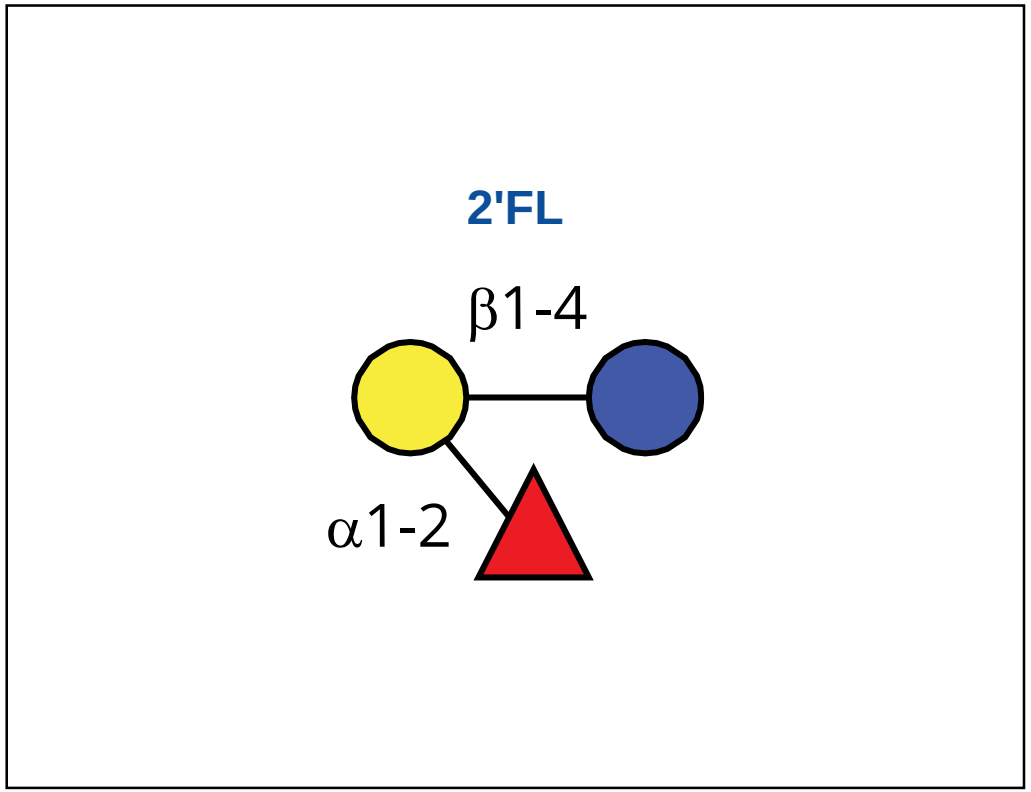
The structure of HMO is unique and linked to their biological activity including effects on microbiota and immune cell populations[4]

2'FL, 2'fucosyllactose; FOS, fructo-oligosaccharide; GOS, galacto-oligosaccharide; HMO, human milk oligosaccharide; LNnT, lacto-N-(neo)tetraose.

1. Sela D. & Mills D. *Trends Microbiol.* 2010.
2. Zivkovic AM, et al. *Proc Natl Acad Sci.* 2011.
3. Bode L. & Jantscher-Krenn E. *Adv Nutr.* 2012.
4. Bode L, et al. *Early Hum Dev.* 2015.
5. Newburg DS et al. *J Nutr*. 2016
6. Austin S. et al. *Nutrients.* 2019

GOS and FOS are less complex structures than HMO[1,2]





2'FL, 2'fucosyllactose; FOS, fructo-oligosaccharide; GOS, galacto-oligosaccharide; HMO, human milk oligosaccharide.

1. Sela D. & Mills D. *Trends Microbiol.* 2010.
2. Bode L. & Jantscher-Krenn E. *Adv Nutr.* 2012.

19/05/2022

Several factors influence the composition, quantity and structure of HMO in human milk

Every mother synthesises and secretes a distinct HMO composition[2]

HMO, human milk oligosaccharide.

1. Miliku K, et al. CHILD study. *Allergy.* 2018.
2. Moossavi S, et al. *Front Pediatr.* 2018.
3. Bode L. Nestle Nutrition Workshop. 2017.

19/05/2022

Stage of lactation influences quantity of HMO in human milk

Colostrum HMO range
20-25 g/L (2-2.5 g/100 mL)[1]

Mature milk HMO range
5-20 g/L (0.5-2.0 g/100 mL)[2]

HMO, human milk oligosaccharide.

1. Coppa GV, et al. *Acta Paediatr.* 1999.
2. Bode L. *Glycobiology*. 2012.

Human milk – the gold standard in infant nutrition

Human milk is uniquely suited both in terms of nutrients and bioactive components required for optimum growth and development of the infant[1]

- A tailor-made food designed to meet all the nutritional needs of the infant.

WHO and UNICEF guidelines recommend exclusive breastfeeding for the first 6 months of life[2,3]

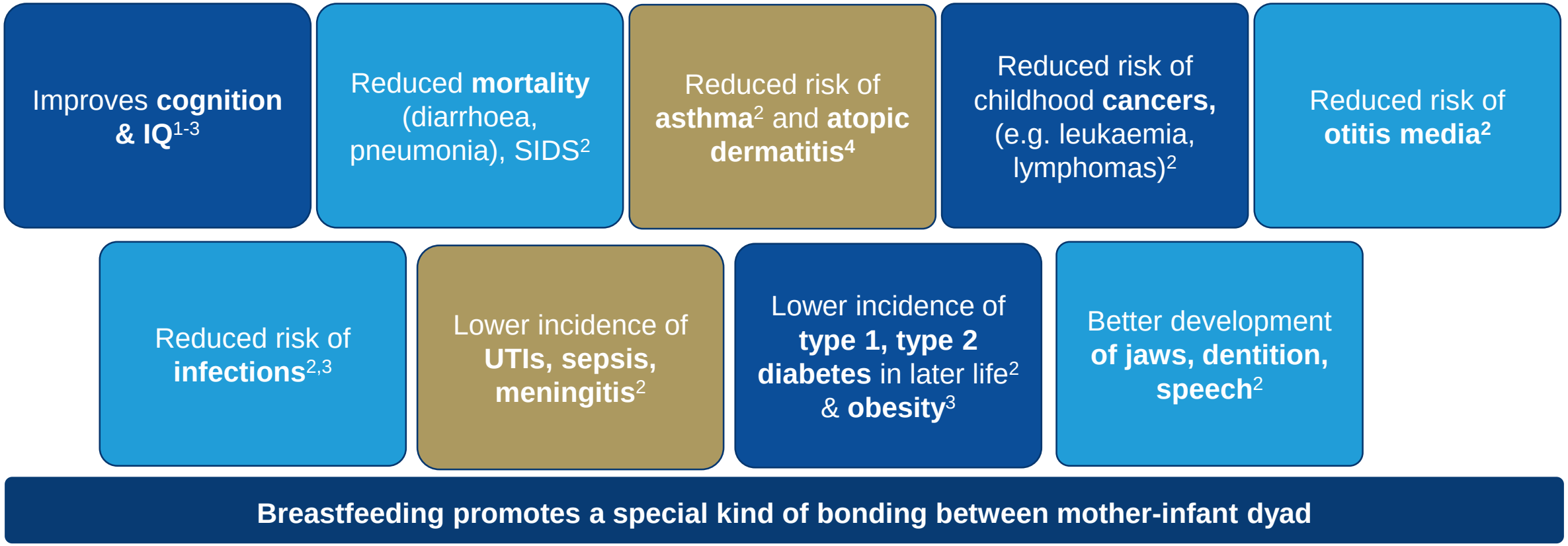
Other mammals produce oligosaccharides, for example, bovine, but have much lower levels and are much less complex[4]

Breastfeeding has numerous benefits including improved IQ and school attendance[5]

IQ, intelligence quotient; UNICEF, United Nations International Children's Emergency Fund; WHO, World Health Organization.

1. Ballard O. & Morrow A. *Pediatr Clin North Am.* 2013.
2. WHO (IYCF). 2009.
3. UNICEF (IYCF). 2011.
4. Bode L. *Nestle Nutr*. Workshop 2017.
5. Victora CG, et al *Lancet*. (The) Breastfeeding Series. 2016.

The numerous benefits of breastfeeding – “Babies are being flushed with HMO”[3]



GI, gastrointestinal; IQ, intelligence quotient; NEC, necrotizing enterocolitis; RTI, respiratory tract infection; SIDS, sudden infant death syndrome; UTI, urinary tract infection.

1. WHO (IYCF). 2009.
2. UNICEF (IYCF). 2011.
3. Victora CG, et al *Lancet*. (The) Breastfeeding Series. 2016.
4. Bode L. *EAPS Presentation.* Paris. 2018.

Summary

HMO are a mix of complex carbohydrates with bioactive features

- ~1% to 2% absorbed – 98% to 99% are non-digested

HMO are the 3rd largest solid component of human milk after lactose and fat

~ 200 different HMO have been identified to date – broadly classified as neutral and acidic

- Neutral HMO represent the largest component ~90%
- 2'FL and LNnT are neutral HMO

HMO are structurally diverse with a lactose backbone

- But more complex than other carbohydrates and more complex than GOS and FOS

Many factors influence the HMO composition

Human milk is the gold standard for human infants

THANK YOU