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HMOs

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Basic Science, Structures and Functions

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Disclosure

- Nothing to declare
- No consultancy for any company

Latest advances - **HMOs and NEC**

Nature Reviews Gastroenterology & Hepatology | News and Views

Caplan MS

Paediatrics: **Are human milk oligosaccharides the magic bullet for necrotizing enterocolitis?**

Nat Rev Gastroenterol Hepatol. 2017 May 17

Latest advances - **HMOs versus Prebiotic Oligosaccharides**

Comstock et al. (Sharon Donovan's group)

Dietary Human Milk Oligosaccharides but not Prebiotic Oligosaccharides
Increase Circulating Natural Killer Cell and Mesenteric Lymph Node Memory T
Cell Populations in **Noninfected and Rotavirus-Infected Neonatal Piglets**

Journal of Nutrition, May 10th, 2017

Latest advances - **Human studies**

- Infants fed a **lower calorie formula with 2'FL** show **growth and 2'FL uptake like breast-fed infants**

Marriage et al. *J Pediatr Gastroenterol Nutr* 2015

- Effects of **infant formula with human milk oligosaccharides** on **growth and morbidity**: a randomized multicenter trial

Puccio et al. *J Pediatr Gastroenterol Nutr* 2017

(2'FL + LNnT)

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(2'FL + LNnT)



Basic Questions

- Are HMOs newly discovered components and what is so unique?
- HMOs and „prebiotic oligosaccharides“?
- HMO functions (GIT, systemic effects)?
- HMO and disease prevention?
- Advantage of supplementing infant formula with HMOs?

History

Before 1900

First description of microorganism (*Lactobacilli*, *Bifidobacteria*)



T. Escherich

Around 1900

Observation

- **different fecal microbiota composition of breast-fed and bottle-fed infants**
- indications: **difference is linked to milk carbohydrates**



E. Moro

1950ff Landmark paper of György: link to *Bifidobacteria*



P. György

PEDIATRICS®

OFFICIAL JOURNAL OF THE AMERICAN ACADEMY OF PEDIATRICS

**A HITHERTO UNRECOGNIZED BIOCHEMICAL DIFFERENCE BETWEEN
HUMAN MILK AND COW'S MILK**

PAUL GYÖRGY

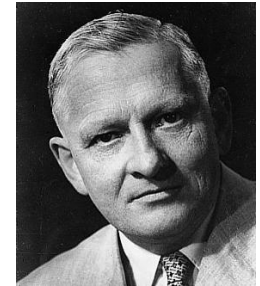
Pediatrics 1953;11:98-108

HMO Pioneers

Richard Kuhn and Paul György

in Heidelberg (1950 ff)

- **First** structural **identification** of HMOs
(2'FL, LNT, LNnT, LNFP I-III, 3'SL, 6'SL etc)
- characterization of **structures** in combination
with functional studies
- in Heidelberg and Philadelphia (1950ff)
characterizing growth factors for *Bifidobacteria*
(„*Lactobacillus bifidus*“)



- Development of **extremely sensitive MS methods**
- **animal studies/in vitro studies** using single HMO to investigate effects on microbiota, inflammation, infections etc.
- first observational studies in **humans** relating a **specific HMO pattern** to diseases
- first **metabolic studies** in **term and preterm infants**

HMOs

- ➔ **largest compositional gap** between human milk and infant formula
- ➔ enormous progress in **HMO availability**
- ➔ beginning of a **new era** investigating single HMOs in **clinical studies**



Macronutrients in mature milk (g/L)



	Human Milk	Bovine Milk
Protein	10	33
Fat	40	40
Lactose	65	47
Oligosaccharides	5 - 15	not present, few in traces only

Kunz und Rudloff *Acta Paediatrica* (1993)

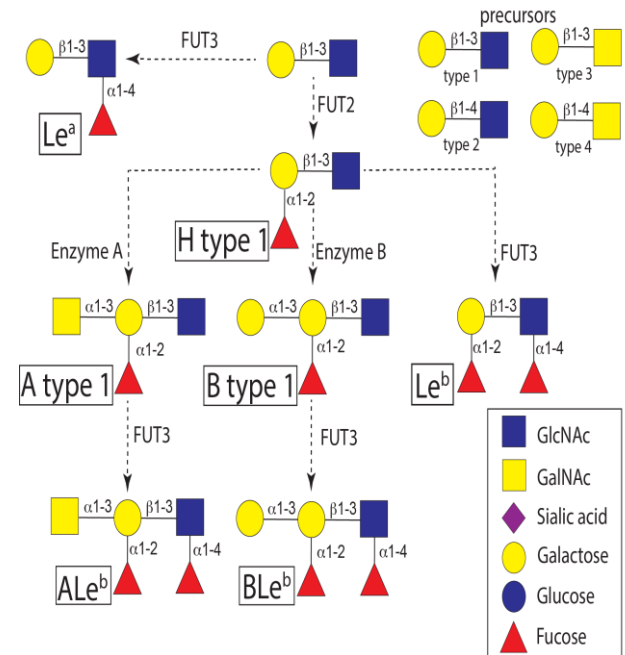
HMO structures

-

What is so unique?

Oligosaccharides in human milk

- Large total amount
- Extremely high structural diversity
- 150 – 200 components identified
- Complex composition predisposes HMOs to **multifunctionality**



Morozov, Hansman, Hanisch, Schroten, Kunz (submitted)

HMO composition

Monosaccharides



Fucose



Neu5Ac



N-Acetyl
Glucosamine

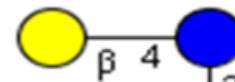


Galactose



Glucose

Lactose as backbone

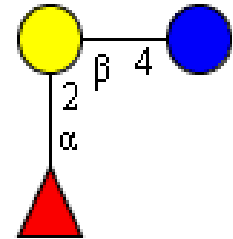


Gal β 1-4Glc

Of interest for supplementation

Neutral components

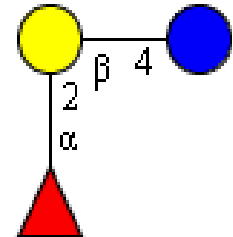
- 2'-Fucosyl-Lactose (2'FL)



Of interest for supplementation

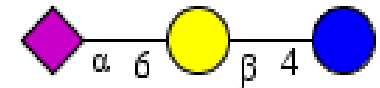
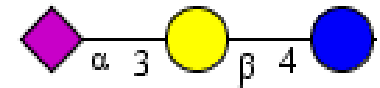
Neutral components

- 2'-Fucosyl-Lactose (2'FL)



Acidic oligosaccharides

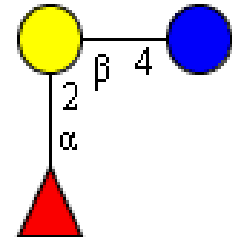
- 3'-Sialyl-Lactose (6'SL)
- 6'-Sialyl-Lactose (3'SL)



Of interest for supplementation

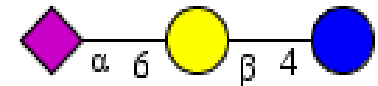
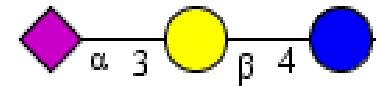
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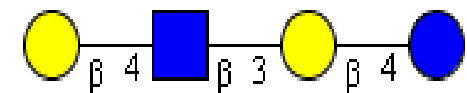
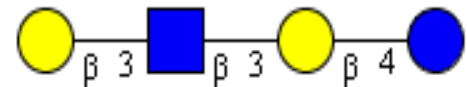
Acidic oligosaccharides

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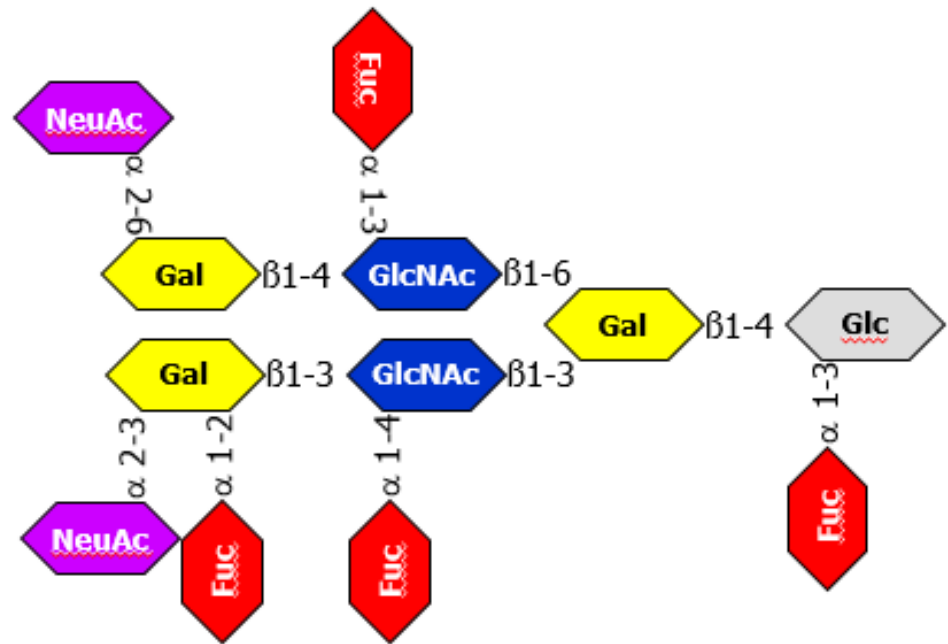
Core Structure

- Lacto-N-Tetraose
- Lacto-N-neo-Tetraose (LNnT)



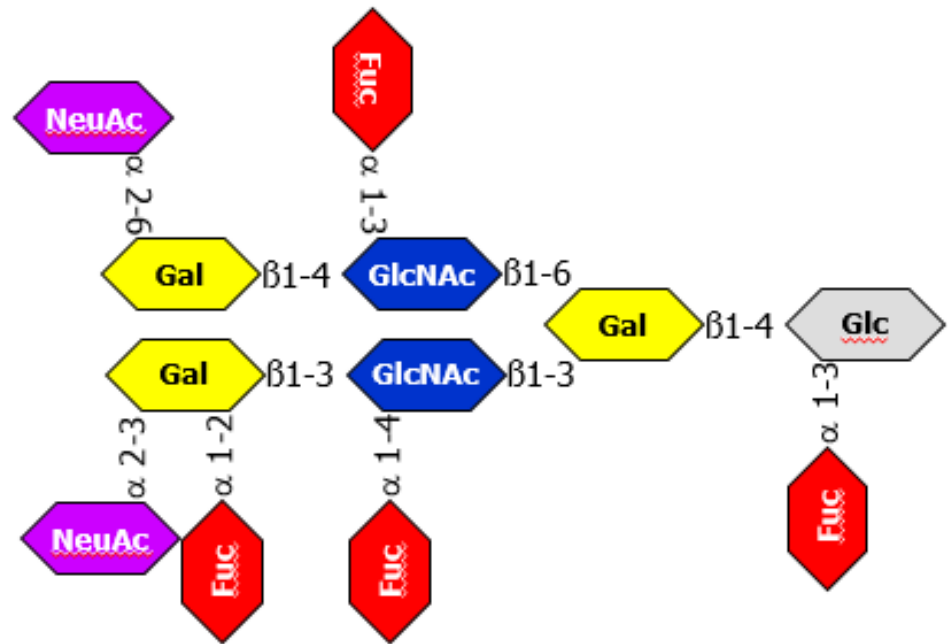
HMOs

- unique structures
- special functions
- unusual metabolism



HMOs

- unique structures
- special functions
- unusual metabolism



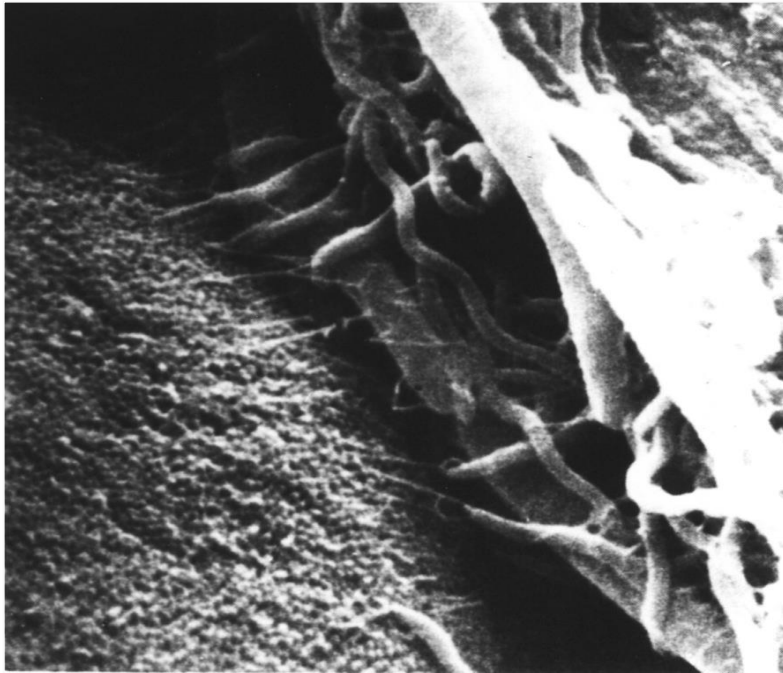
➔ not to compare with any other “prebiotic oligosaccharides” on the market (GOS/FOS etc.)

HMOs

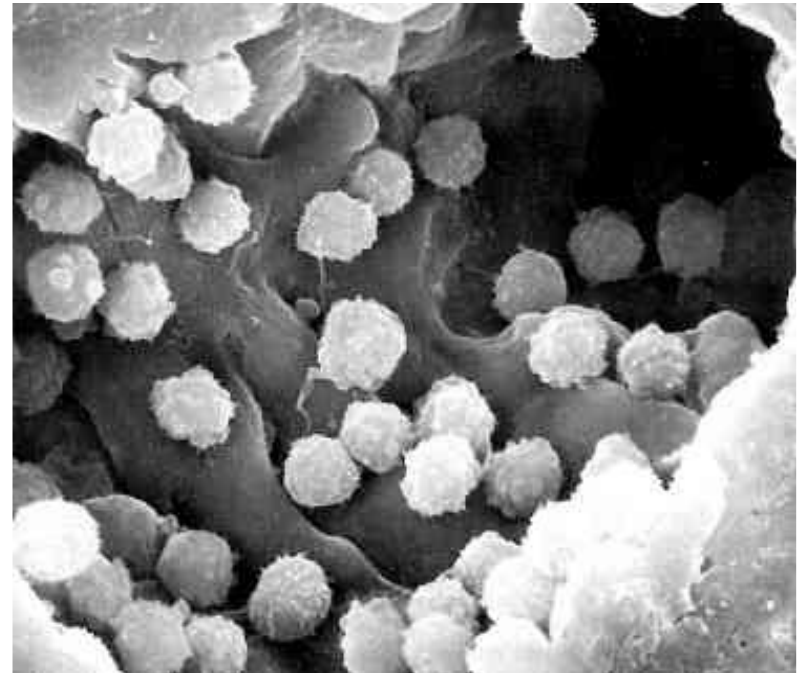
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what are their functions?

Biological Functions of HMO



within the GI tract



systemically

HMOs - Potential Functions in Infants

1. Influence on Microbiota

composition and activity?

Influence of Secretor/Non-Secretor status of the mother?

2. Prevention from Infections

antiadhesive effects for pathogens

3. Interaction with the Immune System

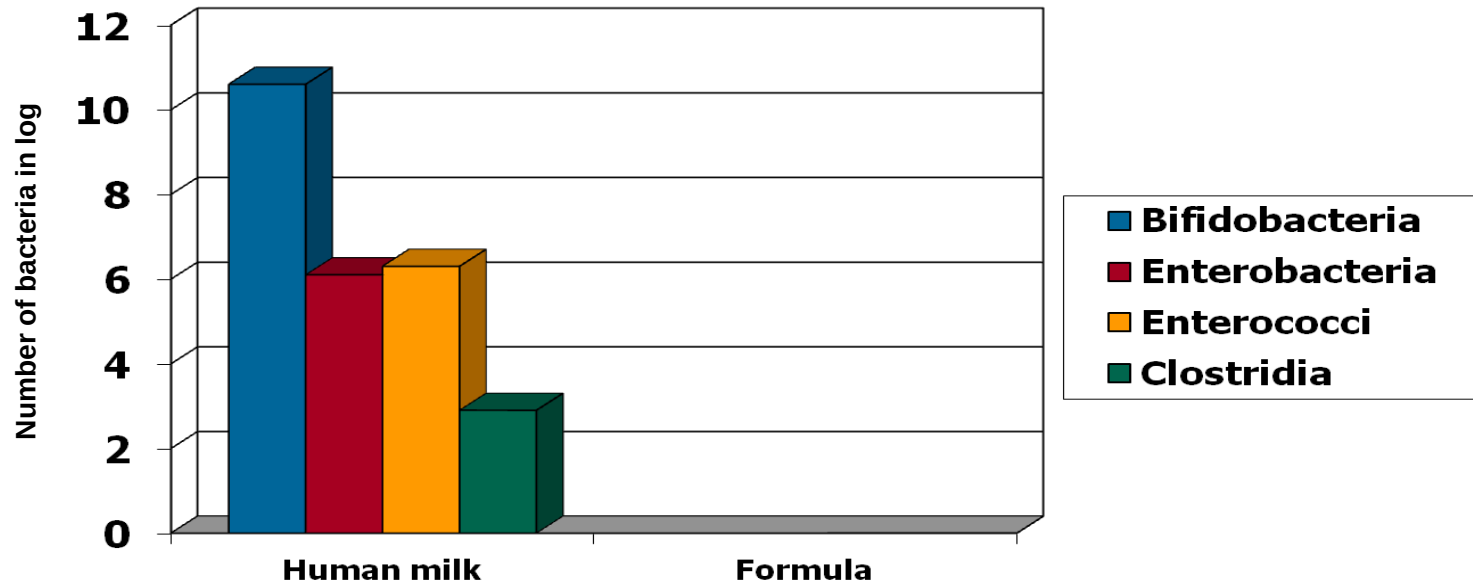
NEC, Morbus Crohn, Colitis ulcerosa; Allergies etc.

**Evidence for the
bidifdogenic effect of
HMOs *in vivo* ?**

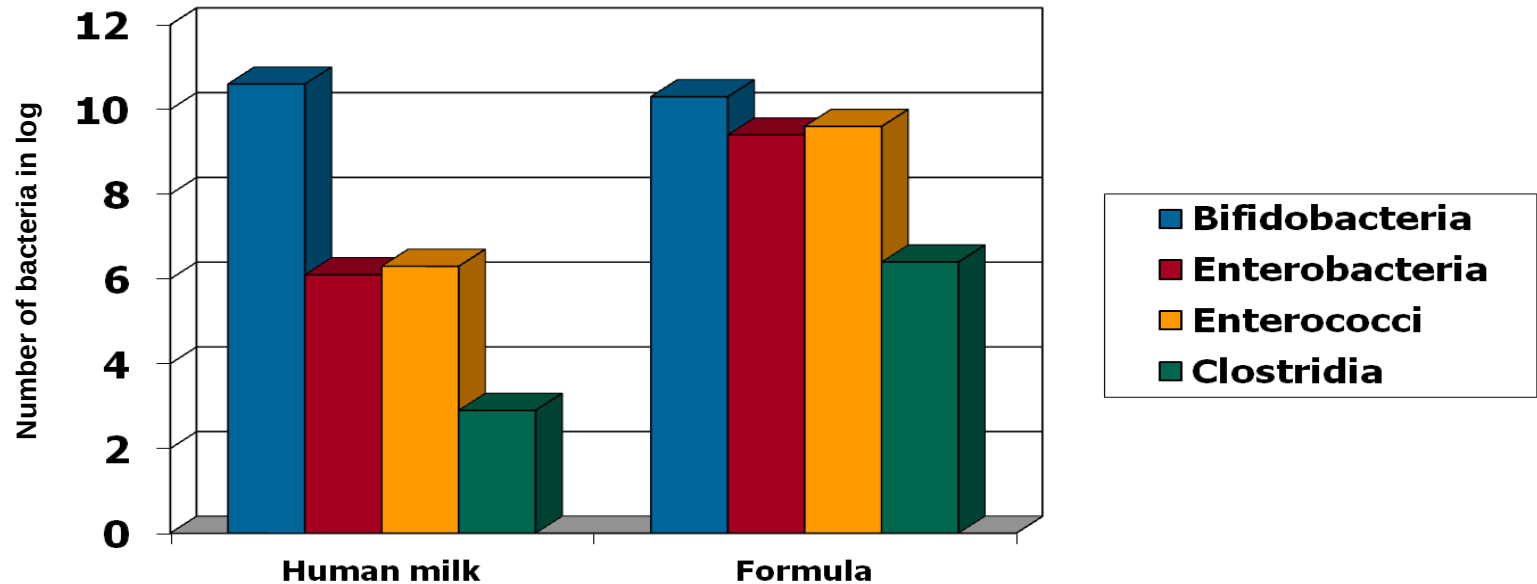


Specific effects or just „growth factors“?

Milk and effects on gut microbiota



Milk and effects on gut microbiota



HMOs - Potential Functions **in Infants**

1. Influence on Microbiota

composition and activity?

Influence of Secretor/Non-Secretor status of the mother?

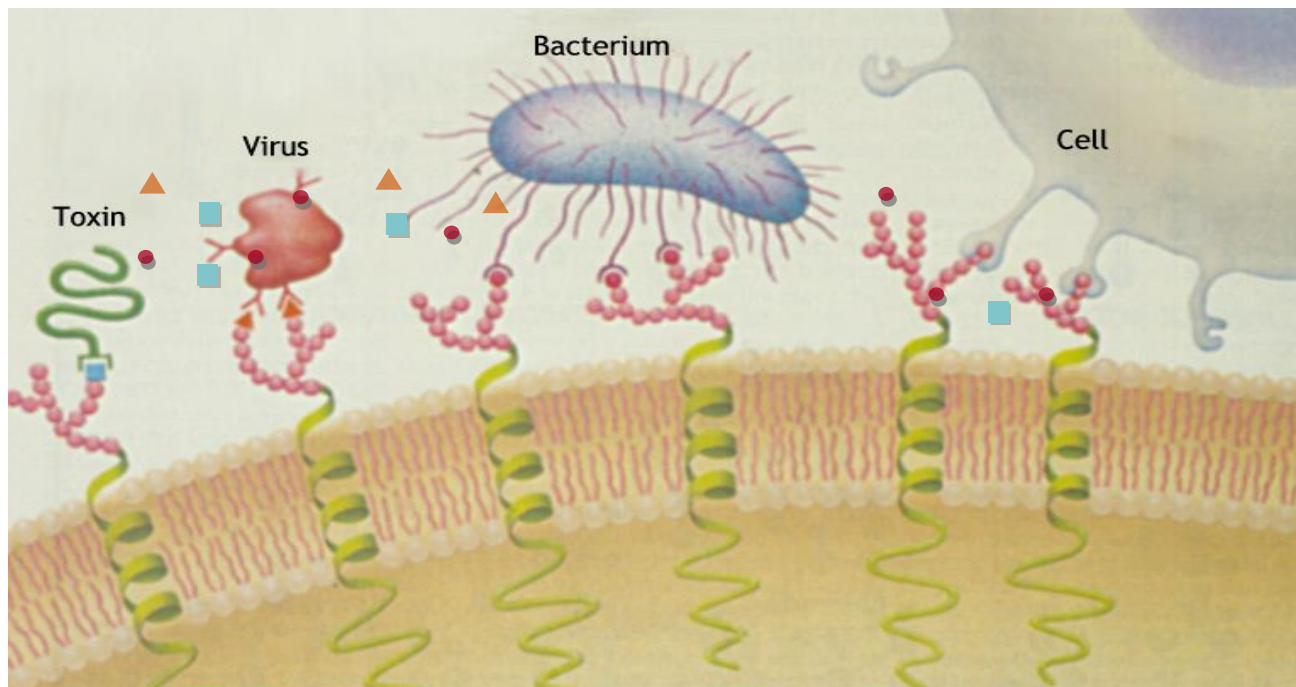
2. **Prevention from Infections**

antiadhesive effects for pathogens

3. Interaction with the Immune System

NEC, Morbus Crohn, Colitis ulcerosa; Allergies etc.

Host Pathogen Interactions through Oligosaccharides



HMO as microbial ligands

Ligand

Fucosylated oligosaccharides

Sialyl(2-3)lactose and glycoproteins

Neutral oligosaccharides (LNT, neo-LNT)

Gal(β 1-4)GlcNAc or Gal(β 1-3)GlcNAc

Fuc1-2Gal epitopes

Sialyl-lactose

Sialylated glycoproteins

Sialyl(2-6)lactose (6'SL)

Sialyl(2-3)lactose (3'SL)

Microorganisms

E. coli (type 1 fimbriae)

E. coli (heat-stable enterotoxin)

Streptococcus pneumoniae

Pseudomonas aeruginosa

Candida albicans

Helicobacter pylori

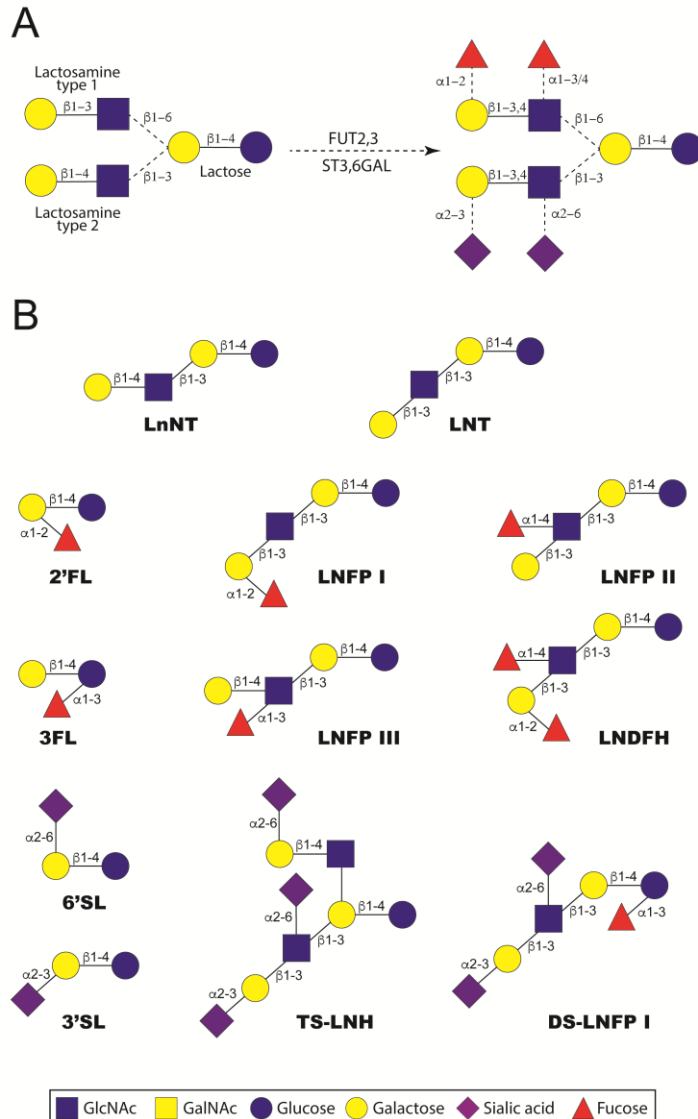
Mycoplasma pneumoniae

Influenza virus A (in humans)

Influenza virus B (in birds)

HMO as microbial ligands

Ligand



Microorganisms

Escherichia coli (type 1 fimbriae)

E. coli (heat-stable enterotoxin)

Streptococcus pneumoniae

Pseudomonas aeruginosa

Candida albicans

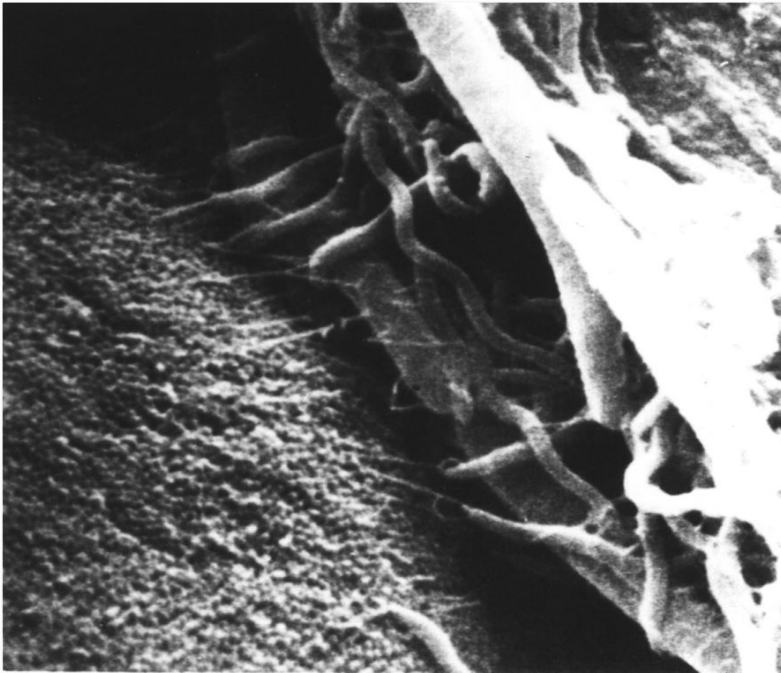
Helicobacter pylori

Mycoplasma pneumoniae

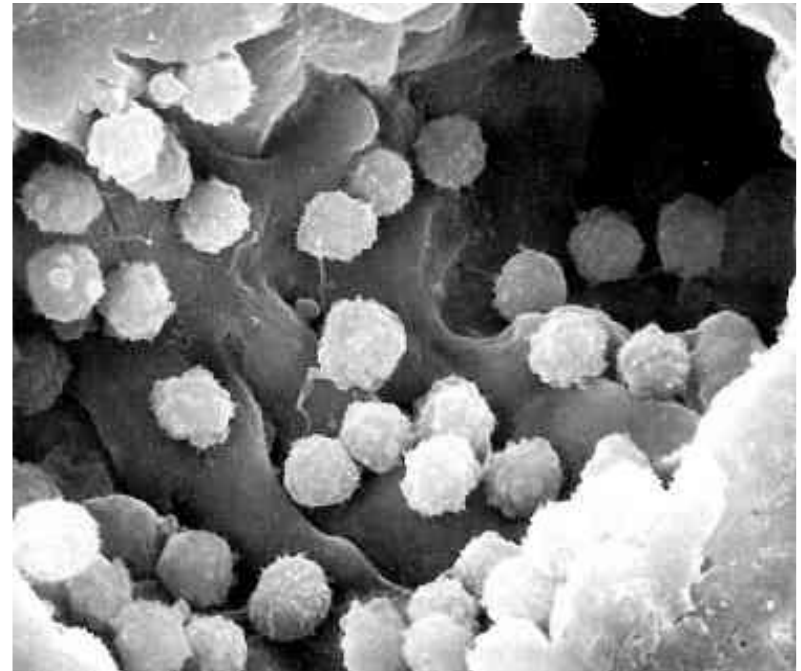
Influenza virus A (in humans)

Influenza virus B (in birds)

Biological Functions of HMO



within the GI tract



systemically

HMOs and Brain

Vázquez E et al.

Oral supplementation of 2'fucosyllactose during lactation improves memory and learning in rats.

J Nutr Biochem 2016

Jacobi et al.

Dietary isomers of sialyllactose increase ganglioside sialic acid concentrations in the corpus callosum and cerebellum and modulate the colonic microbiota of formula-fed piglets.

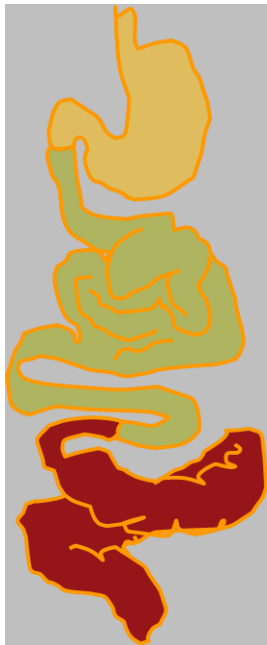
J Nutr 2016

HMOs

-

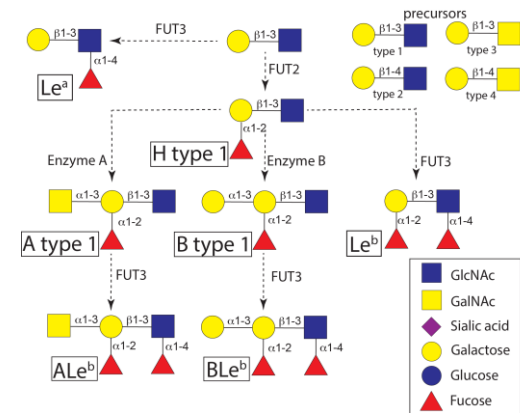
Intake and Metabolism?

HMO digestion and absorption 150 – 200 components



Statement in most publications

- ➔ HMOs resist digestion
- ➔ reach the colon intact
- ➔ available for microbiota



Metabolic fate

Assumption

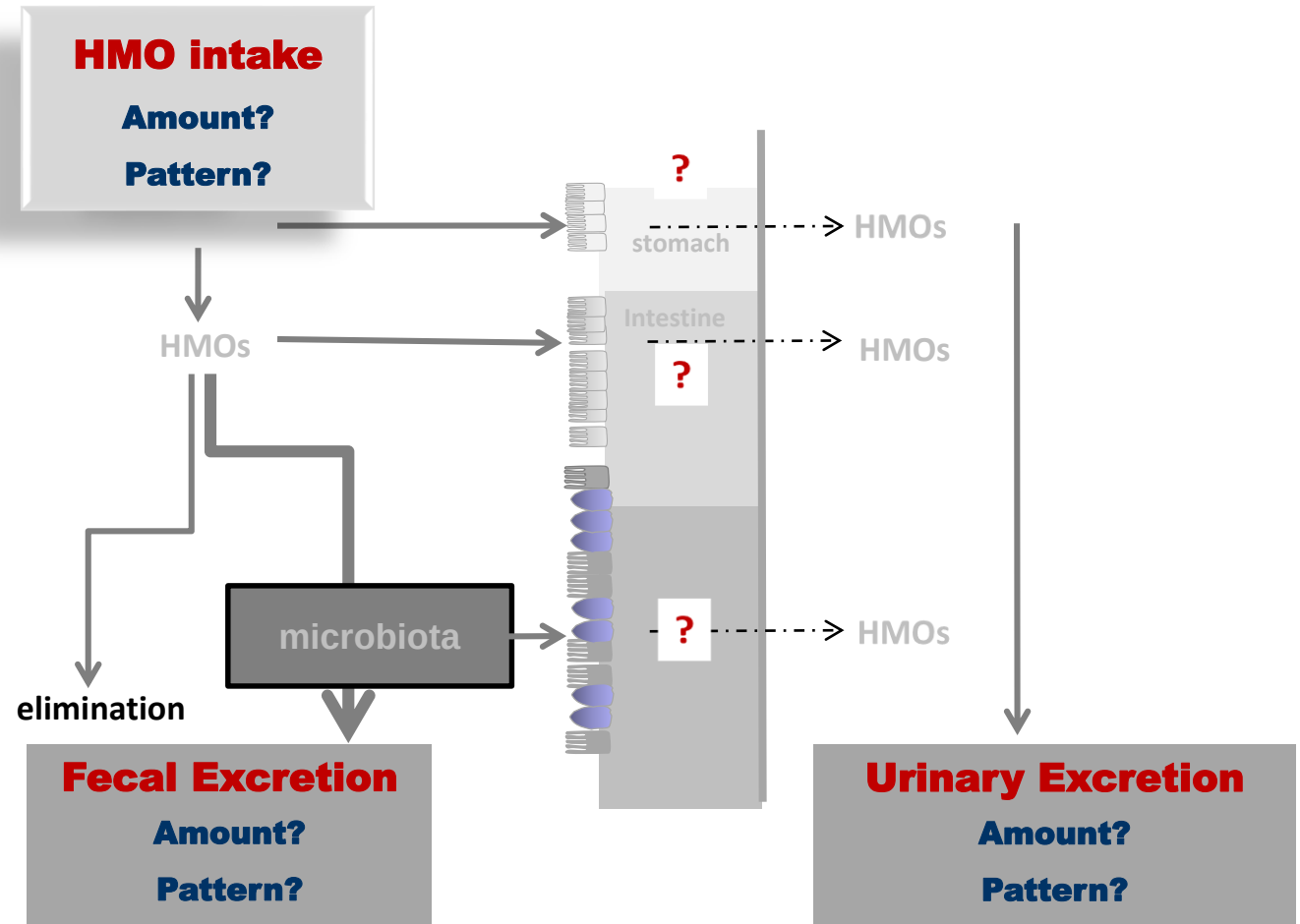
a decrease in fecal HMOs indicates
a preferred utilization by the infants' microbiota



does utilization by the microbiota depend on

- total HMO intake (term vs preterm milk) or
- HMO pattern (secretor versus non-secretor milk)?

Metabolism

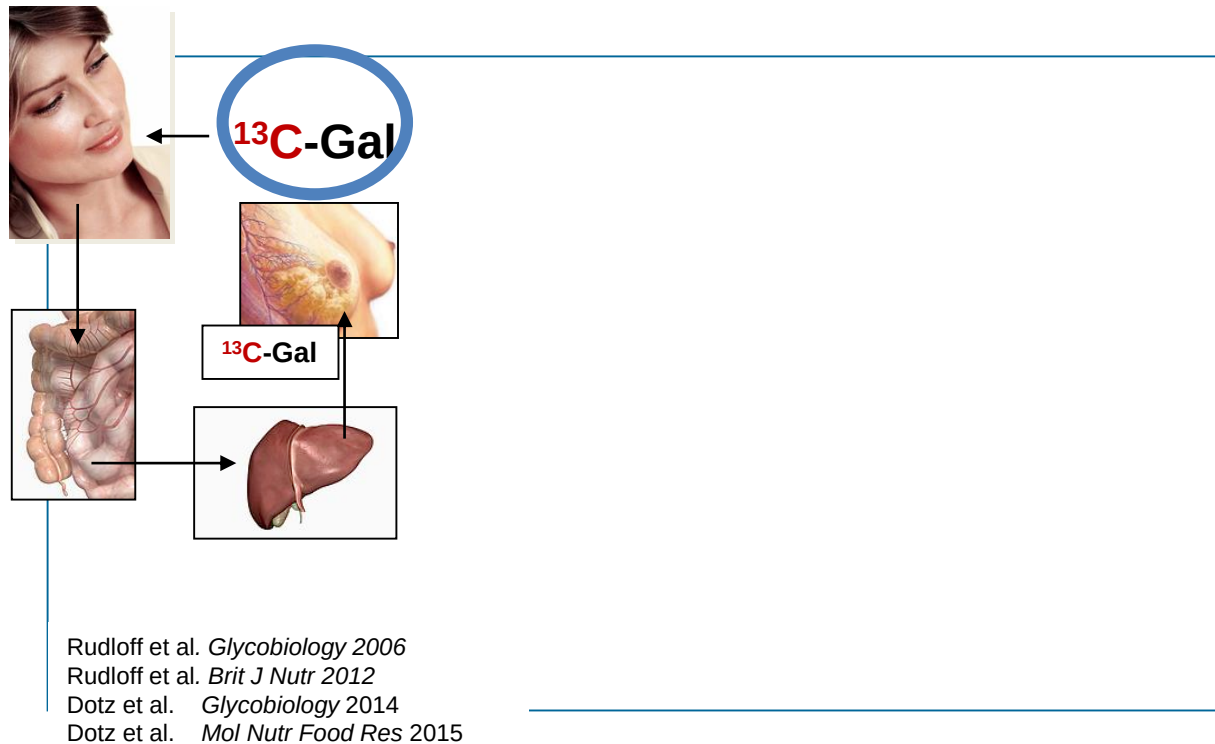


***In vivo* labeling of HMOs**

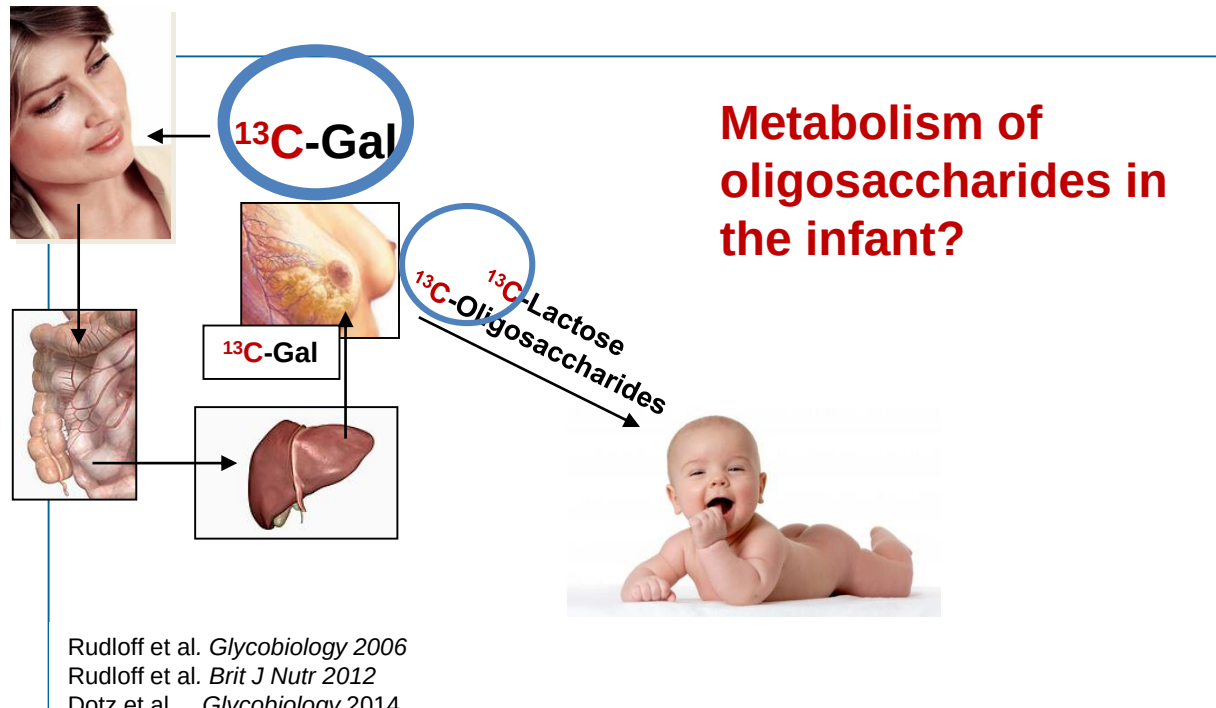


**would alleviate investigations on
the metabolic fate in infants**

Labeling of HMOs with a ^{13}C -Gal bolus



Labeling of HMOs with a ^{13}C -Gal bolus

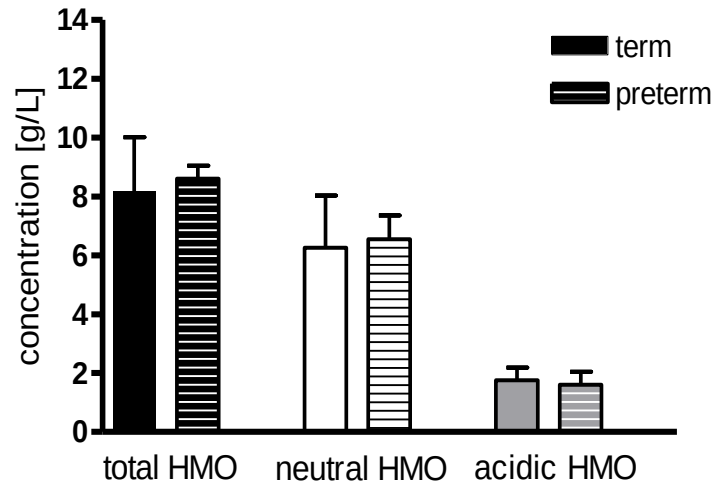


Rudloff et al. *Glycobiology* 2006
Rudloff et al. *Brit J Nutr* 2012
Dotz et al. *Glycobiology* 2014
Dotz et al. *Mol Nutr Food Res* 2015

Concentration of HMOs in mature milk

(Spanish cohort; coll. with Dr. Collado and Prof. Martinez-Costa)

No difference

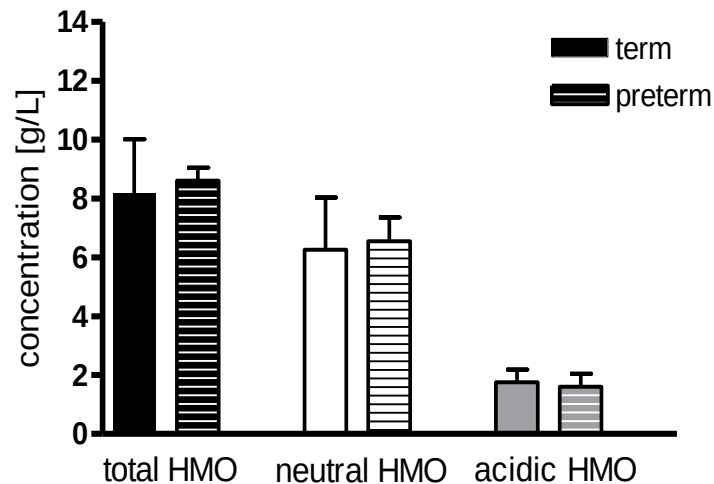


Kunz et al. *JPGN* 2016

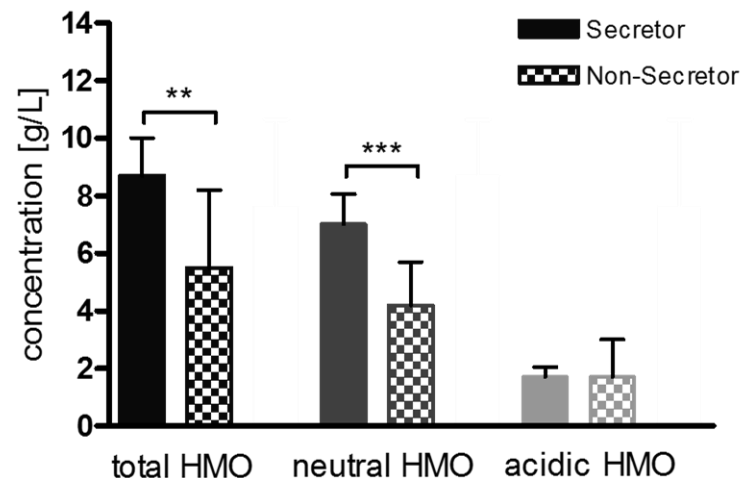
Concentration of HMOs in mature milk

(Spanish cohort; coll. with Dr. Collado and Prof. Martinez-Costa)

No difference



Large differences



Kunz et al. *JPGN* 2016

**Is it important to know
whether an infant receives
Secretor- or Non-Secretor-specific human milk,
i.e. specific HMOs?**



Lewis Blood Group/Secretor status

Secretorstatus/ Lewis Blood Group	% Population	Expression of Fucosyltransferases
Secretor	80 %	α1-2 Fuc-T
Lewis (a ⁺ b ⁻) Non-Secretor	20 %	α1-3/4 Fuc-T
Lewis (a ⁻ b ⁺) <i>Secretor</i>	70 %	α 1-2 Fuc-T α 1-3/4 Fuc-T
Lewis (a ⁻ b ⁻) <i>Secretor</i>	10 %	α 1-2 Fuc-T

Background - Secretor status und Outcomes

Effect	Factor	Reference
FUT 2 important genetic factor with influence on the microbiota in Crohn's disease	Non-secretor status	Rausch et al. 2011
Correlation with human norovirus infections	Secretor-positive status	Prasad et al 2014 Schroten et al 2016
Reduction of diarrhea in infants (e.g. <i>Campylobacter</i> diarrhea)	associated with fucosylated oligosaccharides und 2'FL in milk	Morrow et al. 2004
Assoziation with mortality, Gram negative sepsis und NEC in preterm infants	Low- or Non-Secretor -Status	Morrow et al. 2011
Fecal microbial composition in infants	Dependent on Fut 2-status of the mother	Lewis et al 2015

HMO concentration in mature milk

depends on Lewis blood group and secretor status

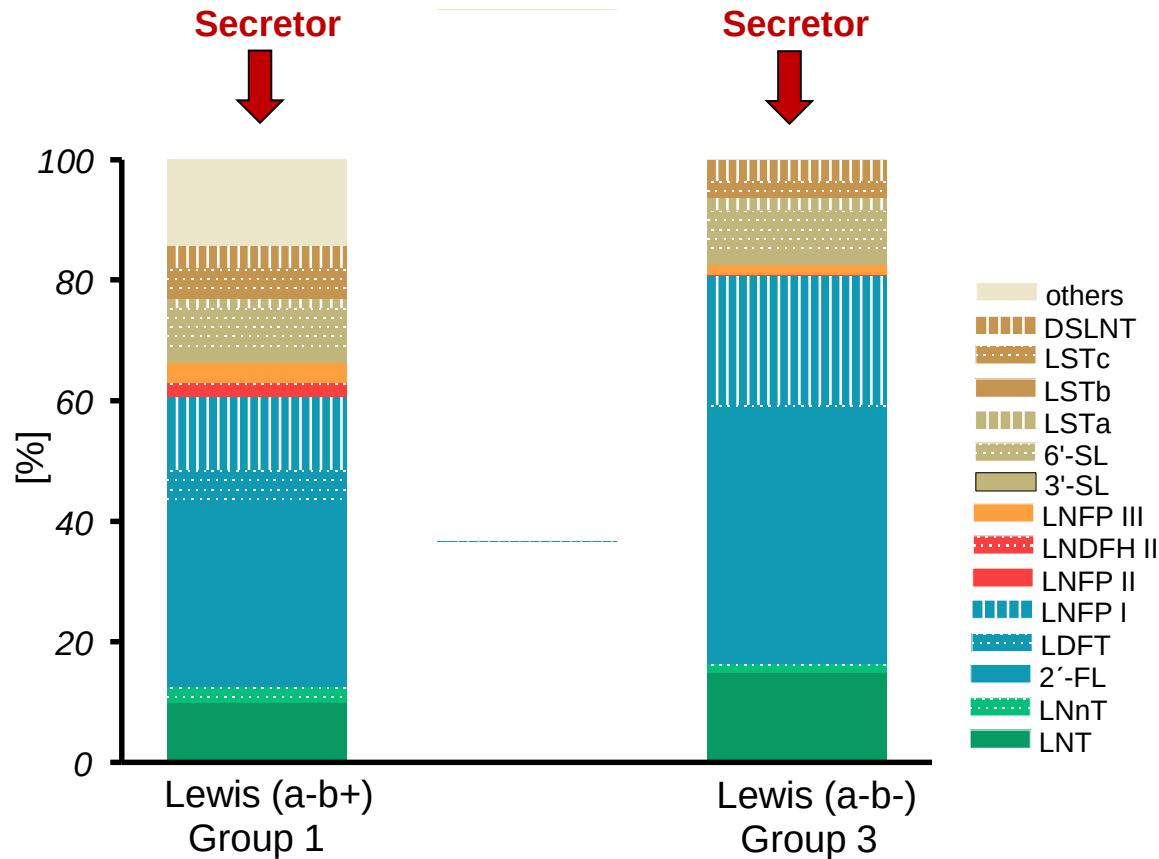


	grams per liter
Oligosaccharides	5 - 15
neutral (fucosylated)	3 - 10
acidic (sialylated)	2 - 5

An infant may receive **completely different single** HMOs!

-

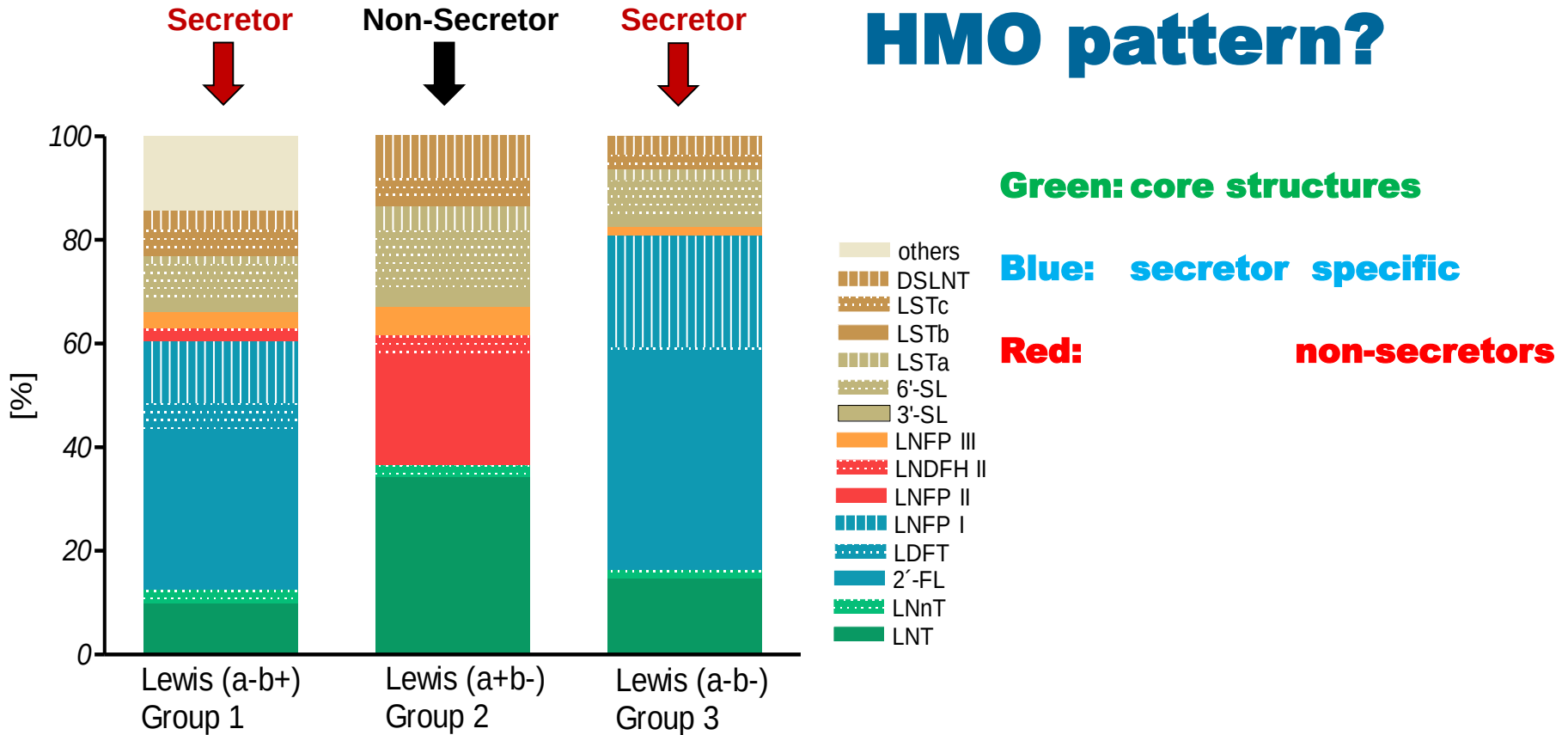
Important for health!?

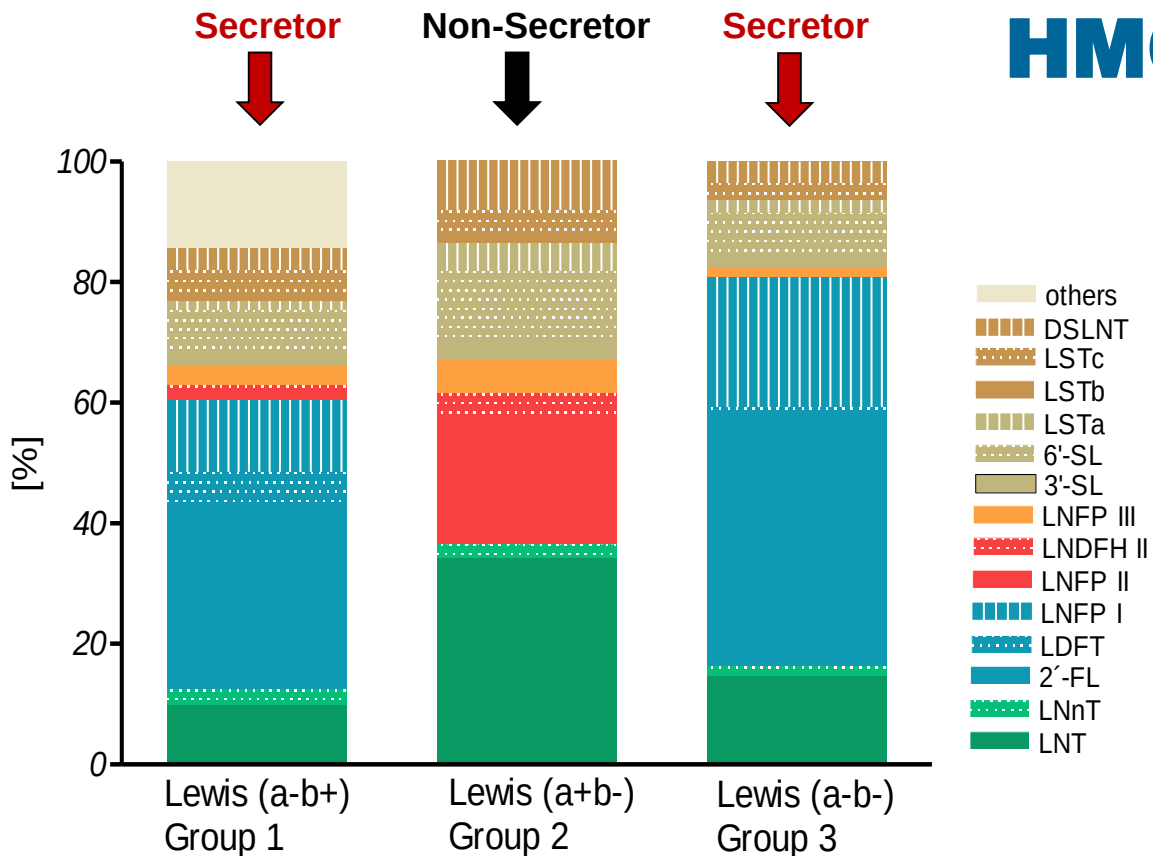


HMO pattern

Green: core structures

Blue: secretor specific





HMO pattern?

Green: core structures

Blue: secretor specific

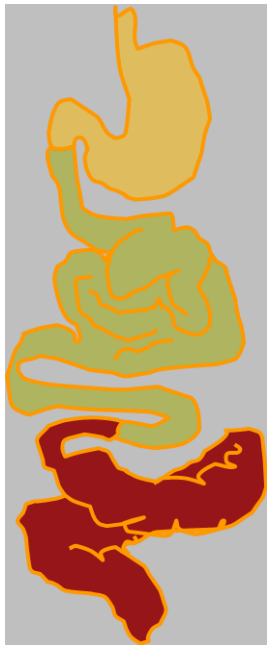
Red: non-secretors

Total Amount (median,QR)

Group 1: 8.7 g/L (8.1-10.1)

Group 2: 5.5 g/L (4.1-8.2)

HMO digestion and absorption



Statement in most publications

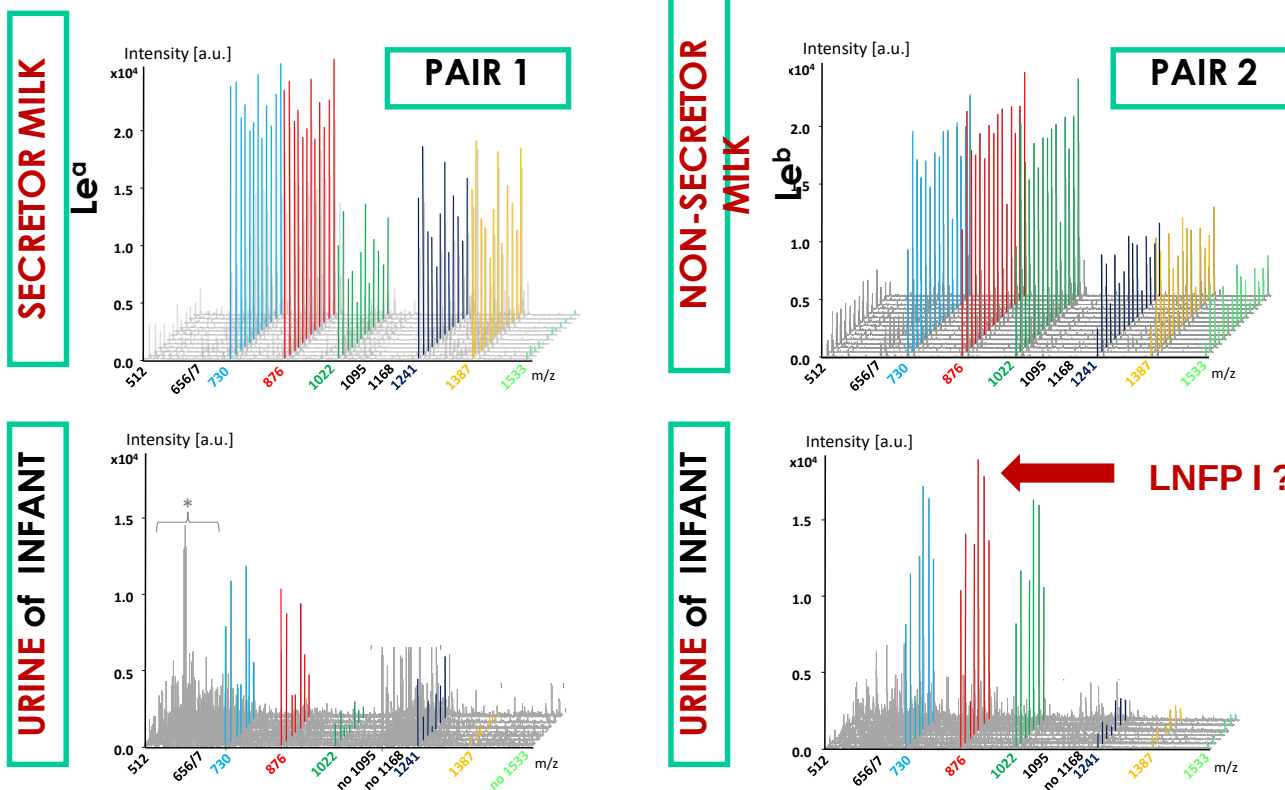
- ➡ HMOs resist digestion
- ➡ reach the colon intact
- ➡ available for microbiota

HMOs in feces?

HMOs in urine?

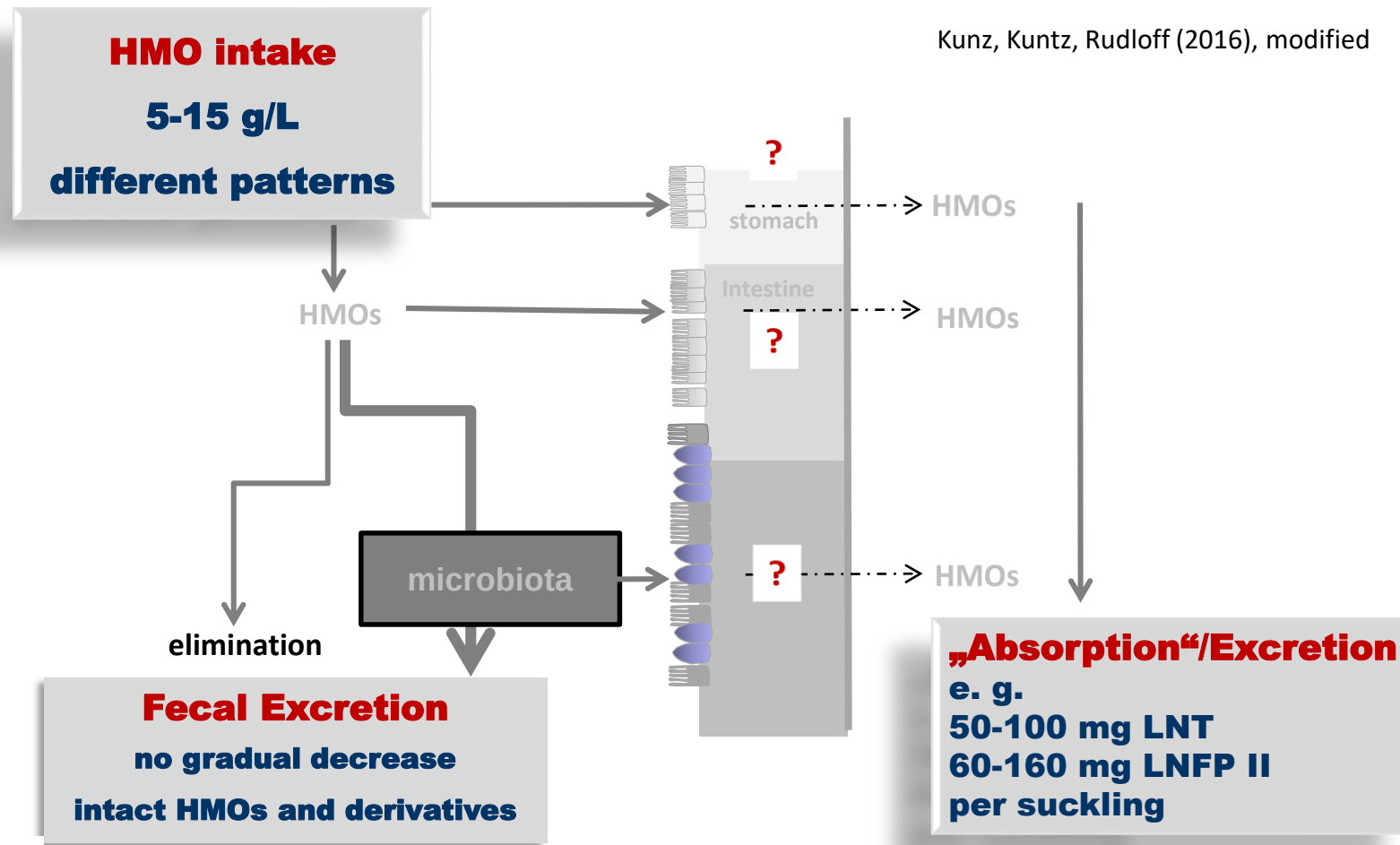


MALDI-MS profiles of mother-child pairs



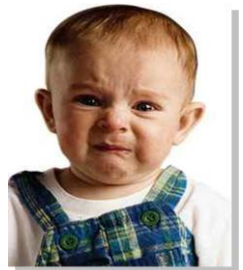
Metabolism

Kunz, Kuntz, Rudloff (2016), modified



HMOs - Conclusion and Main Messages

- **unique components**, inot to compare with other prebiotics



HMOs - Conclusion and Main Messages

- **unique components**, not to compare with other prebiotics
- **structural diversity** of each single HMO predispose them to **specific functions**



HMOs - Conclusion and Main Messages

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- **structural diversity** of each single HMO predispose them to **specific functions**
- **few HMOs** ($n = 5 - 7$) **comprise the majority** of all structures



HMOs - Conclusion and Main Messages

- **unique components**, not to compare with other prebiotics
- **structural diversity** of each single HMO predispose them to **specific functions**
- **few HMOs** ($n = 5 - 7$) **comprise the majority** of all structures
- potential **functions**: (influence of Secretor/Non-Secretor status)
 - **gastrointestinal health**
 - **inflammation and infection**
 - **immune system**



HMOs - Conclusion and Main Messages

- **unique components**, not to compare with other prebiotics
- **structural diversity** of each single HMO predispose them to **specific functions**
- **few HMOs** ($n = 7 - 10$) **comprise the majority** of all structures
- potential **functions**: (influence of Secretor/Non-Secretor status)
 - **gastrointestinal health**
 - **inflammation and infection**
 - **immune system**
- **single HMO components** should carefully be investigated



HMOs - Conclusion and Main Messages

concerning 2'FL and LNnT

- Components are **identical to those in human milk**
- Components accepted by the Regulatory Authorities as **safe**
- Tolerance : no negative effects reported
- **Primary outcome** of reported studies: **growth rate!**
(comparable to breastfed infants)

Acknowledgements

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