



novonesis

# The science behind Novonesis HMOs

Healthy Growth. Born from Science.™

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# 5 HMO Mix

Healty Growth.  
Born from Science.™

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# Clinical studies on the supplementation of manufactured human milk oligosaccharides: A systematic review

HMOs were studied clinically in infants (both healthy and diseased), children and adults. Safety and tolerance consistently shown across all study populations

HMOs shifted outcomes towards those observed in breastfed infants, including stool characteristics, gut microbiome, immune markers and health state

In non-infant populations, HMO supplementation positively impacted gut health and the immune system

## Executive summary:

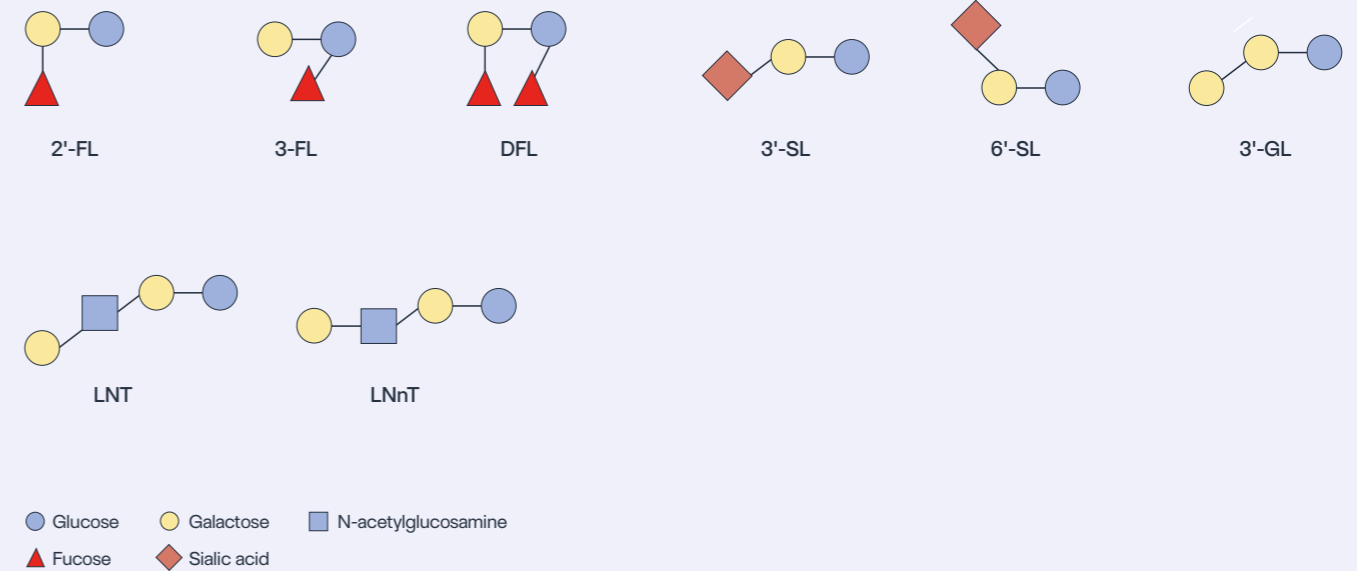
The systematic review is based on 26 published clinical trials on HMOs (12 studies in healthy infant, 5 in infants with medical indications, 2 in children and 7 in adults) for evaluating health outcomes of clinical trials that investigated supplementations of HMOs.

It is shown that HMO supplementation is safe and well-tolerated among all tested populations and health states. Studies also reported indications for health benefits associated with HMO intake including microbiome modulation, immune and gut health. Many outcomes for formula-fed infants shifted in the direction of breastfed infants when supplying with HMOs. Novonesis is continuing research on microbiome analysis in clinical trials and in vitro study on immune cells, further demonstrating the beneficial effects of HMOs in infants.



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## Tested HMOs in clinical studies



\* Controversy as to whether 3'-GL is categorized as an HMO.

## Summarized clinical trials that investigated HMO supplementation in infants

		Gut Microbiome	Metabolites	Stool characteristics	Immune markers	Health state
1 HMO	0.2 g/L LNnT	-	-	-	-	-
	0.2 g/L 2'-FL + 2.2 g/L GOS 1.0 g/L 2'-FL + 1.4 g/L GOS	-	Microbiota-derived metabolites modulated Plasma secondary bile acids modulated	-	Immune markers close to breastfed	+
	1.0 g/L 2'-FL + <i>B. lactis</i>	-	-	-	-	+
	1.0 g/L 2'-FL + <i>L. reuteri</i>	<i>Bifidobacteria</i> ↗ <i>C. difficile</i> ↘	-	Fecal pH ↘	-	-
	1.0 g/L 2'-FL	-	Metabolic capacity modulated	-	-	-
2 HMOs	1.0 g/L 2'-FL + 0.5 g/L LNnT	<i>Bifidobacteria</i> ↗ <i>Escherichia</i> and <i>Streptococcus</i> ↘	SCFA modulated acetate ↗ butyrate ↘	Softer stools at 2 months	-	+
5 HMOs	5.75 g/L 2'-FL + 3-FL + LNT + 3'-SL + 6'-SL	<i>Bifidobacteria</i> ↗ Microbiome composition shifted	Metabolic capacity modulated	Stool frequency ↗ Consistency softened Closer to breastfed	-	+
	1.5 & 2.5 g/L 2'-FL + DFL + LNT + 3'-SL + 6'-SL	Infant-type <i>Bifidobacterium</i> ↗ <i>C. difficile</i> ↘	SCFA modulated acetate ↗	Fecal pH	slgA ↗ AAT-1 ↘	-

# Clinical trial about the tolerability and safety of a 5 HMO Mix in healthy-term infants

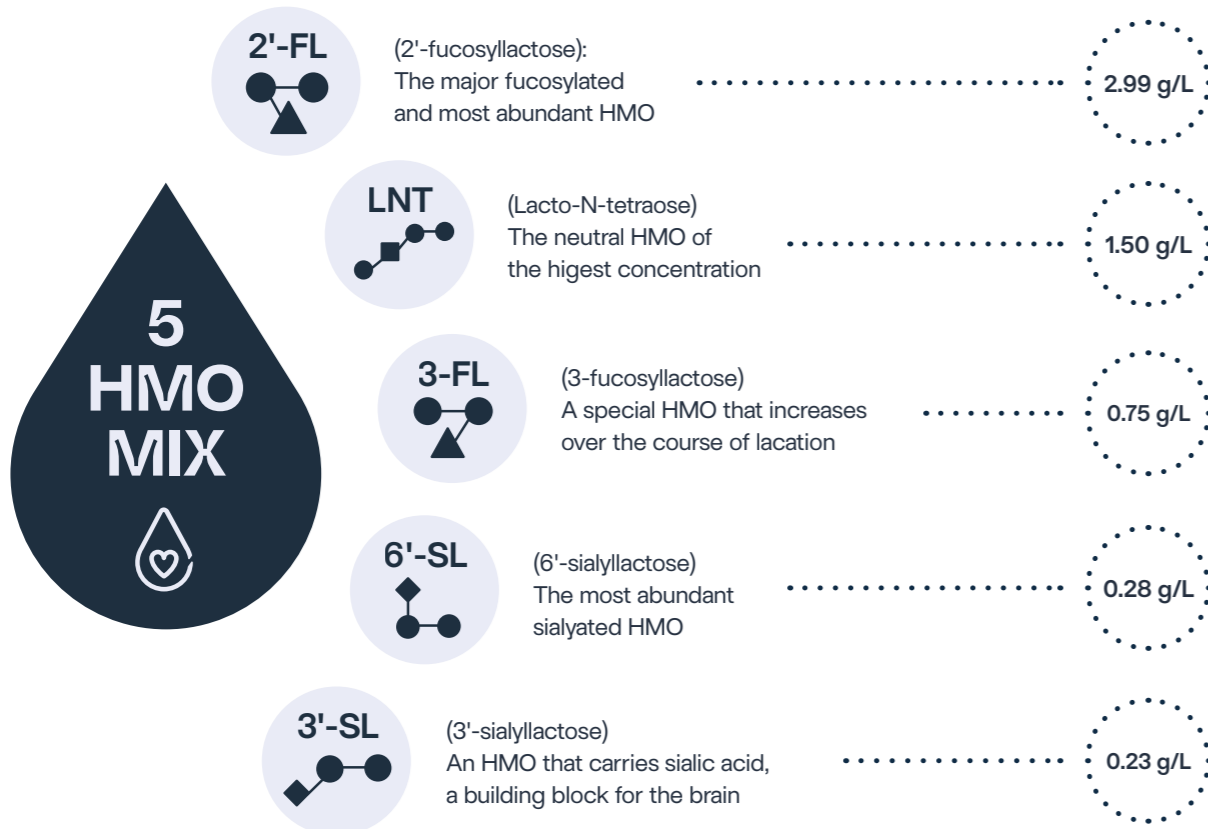
Study on 5 HMO Mix at 5.75 g/L with 2'-FL, 3-FL, LNT, 3'-SL and 6'-SL

Evidence of the safety, tolerability of the 5 HMO Mix and effects on more frequent and softer stools

Follow-up analyses for effects of HMOs and breastfeeding on the gut microbiome development

## Structures in the 5 HMO Mix

Each HMO in Novonesis 5 HMO Mix is reflecting the natural concentrations of the HMOs in breast milk.



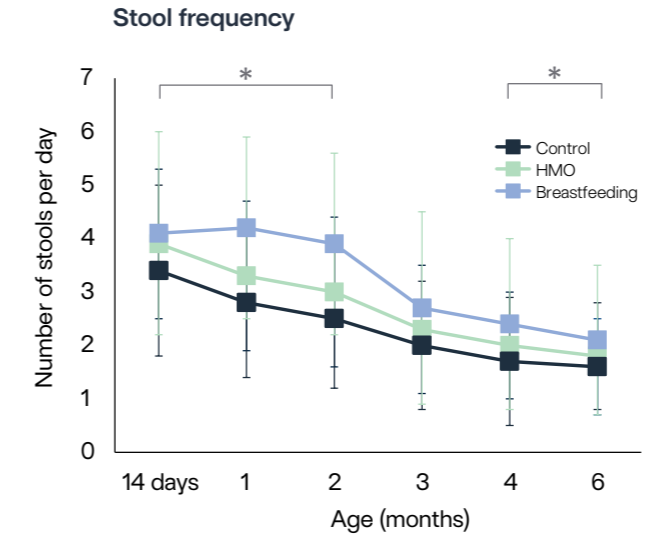
Study aim: Evaluate the safety, tolerability, and health benefits of the 5 HMO Mix

## Executive summary:

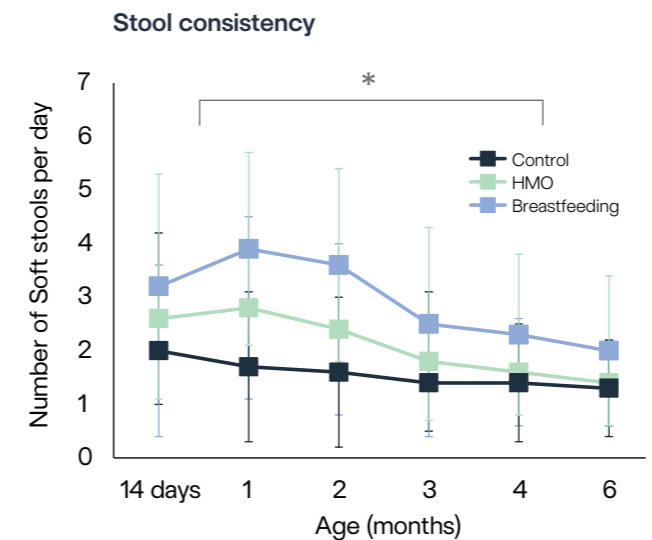
This is the first randomized, controlled, clinical multi-centric study assessing the safety, tolerability, and effect on growth of formula supplemented with a 5 HMO blend at 5.75 g/L. It comprises the structurally diverse HMOs 2'-FL, 3-FL, LNT, 3'-SL, and 6'-SL.

341 healthy infants (<14 days) were allocated into three groups: feeding formula with HMOs, formula without oligosaccharides, and exclusively breastfeeding for 4 months.

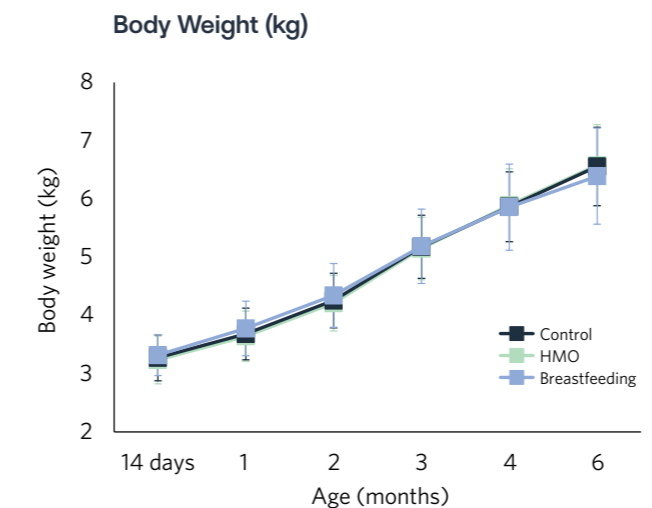
The study showed that feeding infant formula containing a 5 HMO blend is safe, well-tolerated and modulated stooling pattern. Further, the effect of HMOs on the gut microbiome is currently under investigation.



Stools per day differed significantly in the order of breastfed group > HMO group > control group.



HMO group showed softer stools than control group. Breastfed infants showed consistently softer stools.



The HMO group showed adequate growth, in line with the control and breastfed groups.



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# Infant formula supplemented with five human milk oligosaccharides shifts the fecal microbiome of formula-fed infants closer to that of breastfed infants

Study on infant formula supplemented with 5 HMO Mix at 5.75 g/L with 2'-FL, 3-FL, LNT, 3'-SL and 6'-SL for analysis of infant fecal microbiome

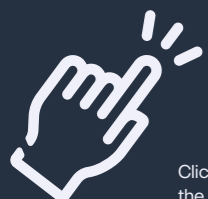
5 HMO Mix cohort has an increased abundance of *bifidobacteria* and lower of opportunistic pathogens compared to formula-fed control

5 HMO Mix supplement modulates the microbiome in shaping microbiome compositions and changing metabolic module, which shifts the fecal microbiome towards breastfed infants

## Executive summary:

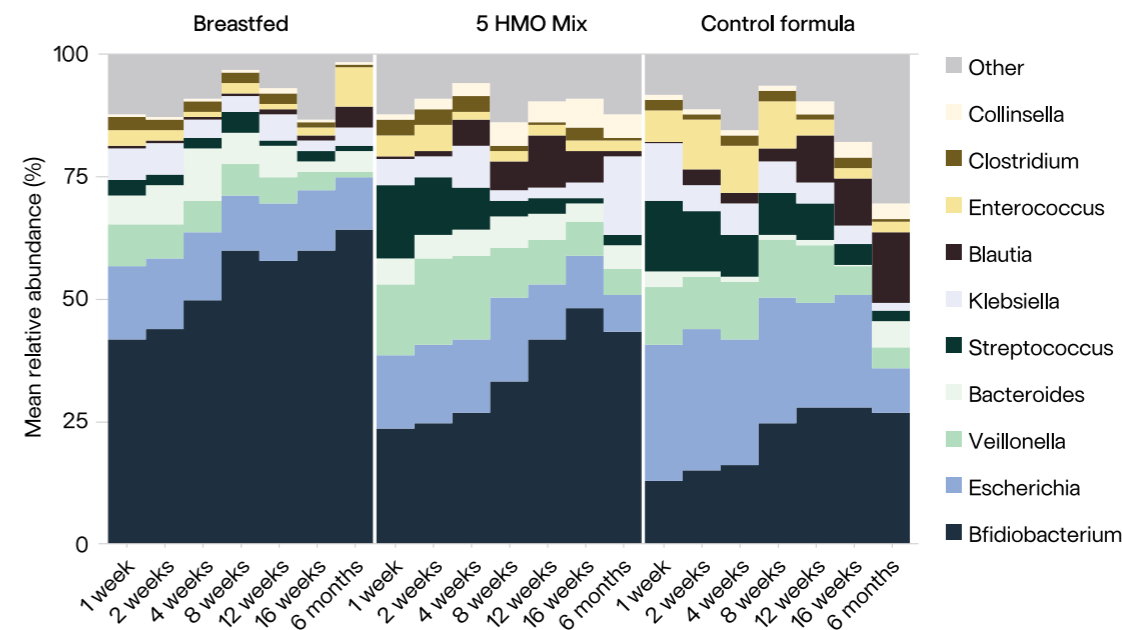
This is the first clinical study focusing on infant formula supplemented with 5 HMO Mix (2'-FL, 3-FL, LNT, 3'-SL and 6'-SL) at 5.75 g/L for understanding of infant fecal microbiome. 311 healthy infants (<14 days) were categorized into 3 groups including feeding infant formula with or without 5 HMO Mix and breastfeeding. In this study, it shows that 5 HMO Mix supplement group supports the development of the early-life microbiome and shifts its composition closer to breastfed infants compared with infant formula control.

Further, the supplements also changes the metabolic functional modules of the microbiome. These findings could support unraveling linkage between infant microbiome, immune development and long-term health effects in the future.



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## Infant formula with 5 HMO Mix shifts the gut microbiome closer to that of breastfed infants



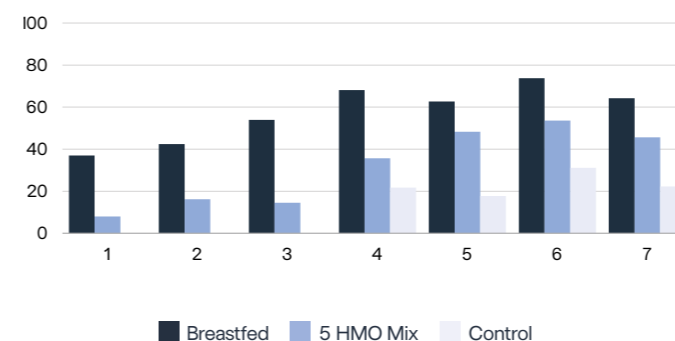
### 5 HMO Mix specifically influences the microbiome

- Higher Bifidobacteria abundance
- Lower opportunistic pathogen abundance

### 5 HMO Mix specifically influences the microbiome

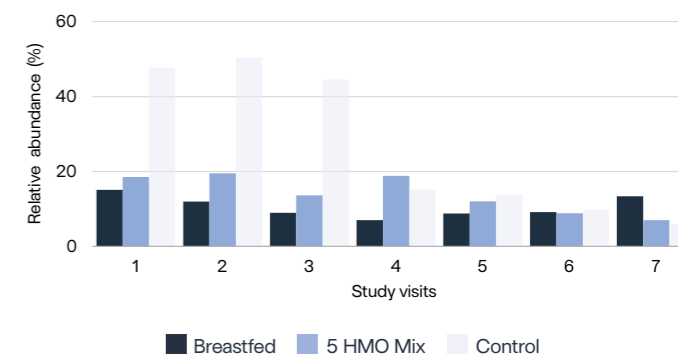
#### Favorable changes in bacterial composition

*Bifidobacteria*



- Higher *bifidobacteria* abundance in HMO group than formula-fed control
- HMO group shifted microbiome towards breastfed

#### Potentially undesirable bacteria



- Lower opportunistic pathogen abundance

# Variation in the conservation of species-specific gene sets for HMO degradation and its effects on HMO utilization in *bifidobacteria*

## Understanding bacterial interactions with HMOs

Assessment of 130 *bifidobacteria* strains from 10 species for their growth on HMOs (2'-FL, 3-FL, LNT, LNnT, 3'-SL, 6'-SL)

Great variability in HMO-degrading gene sets influences the different utilization capabilities between bifidobacterial species

Structure-specific differences affect HMO utilization, with LNT identified as the superior growth substrate utilized by many bifidobacterial species

### Executive summary:

This study assessed 130 bifidobacterial strains to identify the gene sets involved in HMO degradation and their impact on HMO utilization. *B. infantis* stands out as the most efficient HMO utilizer due to its comprehensive set of conserved HMO-degrading genes. In contrast, other species like *B. breve* and *B. bifidum* show varying degrees of HMO utilization.

The study also highlights that LNT is the most widely used HMO among various bifidobacterial strains, underscoring its importance as a key nutrient in shaping the gut microbiota of breastfed infants. These findings provide insights into the adaptive strategies of bifidobacterial species in utilizing this important class of HMOs, which have significant implications for the gut microbiome and infant health.

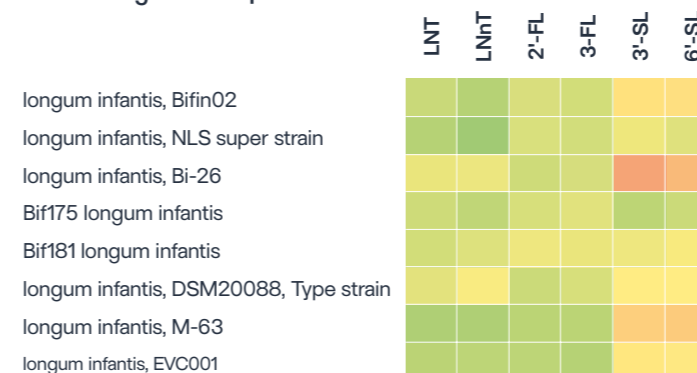


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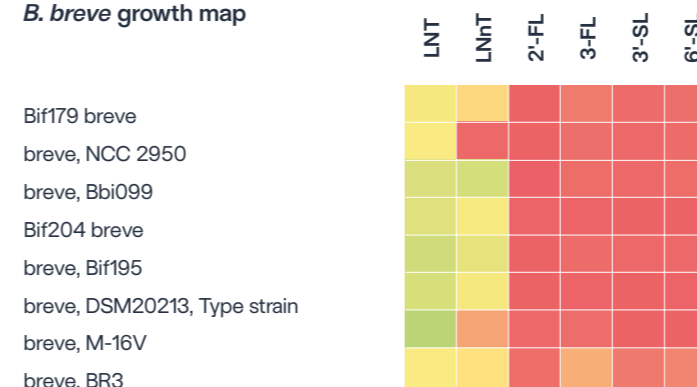
## Distinct differences in *bifidobacteria* growth on HMOs

- B. infantis* grew on all HMOs but lower on SLs.
- B. breve* grew on LNT and LNnT, preferred growth on LNT.
- B. Bifidum* grew well on LNT and LNnT but lower on other HMOs.

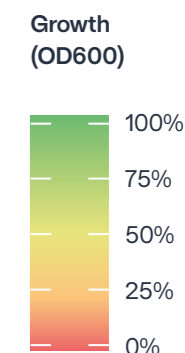
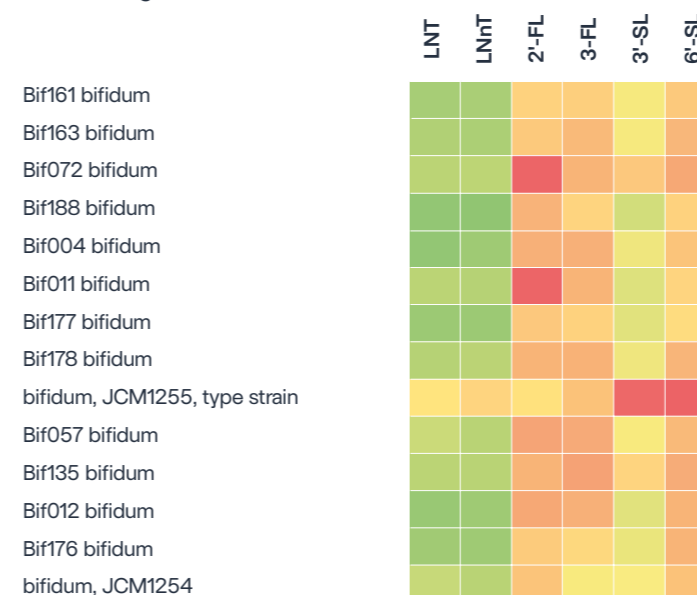
*B. infantis* growth map



*B. breve* growth map



*B. bifidum* growth map



# Human milk oligosaccharides differentially support gut barrier integrity and enhance Th1 and Th17 cell effector responses *in vitro*

Study indicates that HMOs play important role in supporting intestinal barrier integrity and modulate immune system



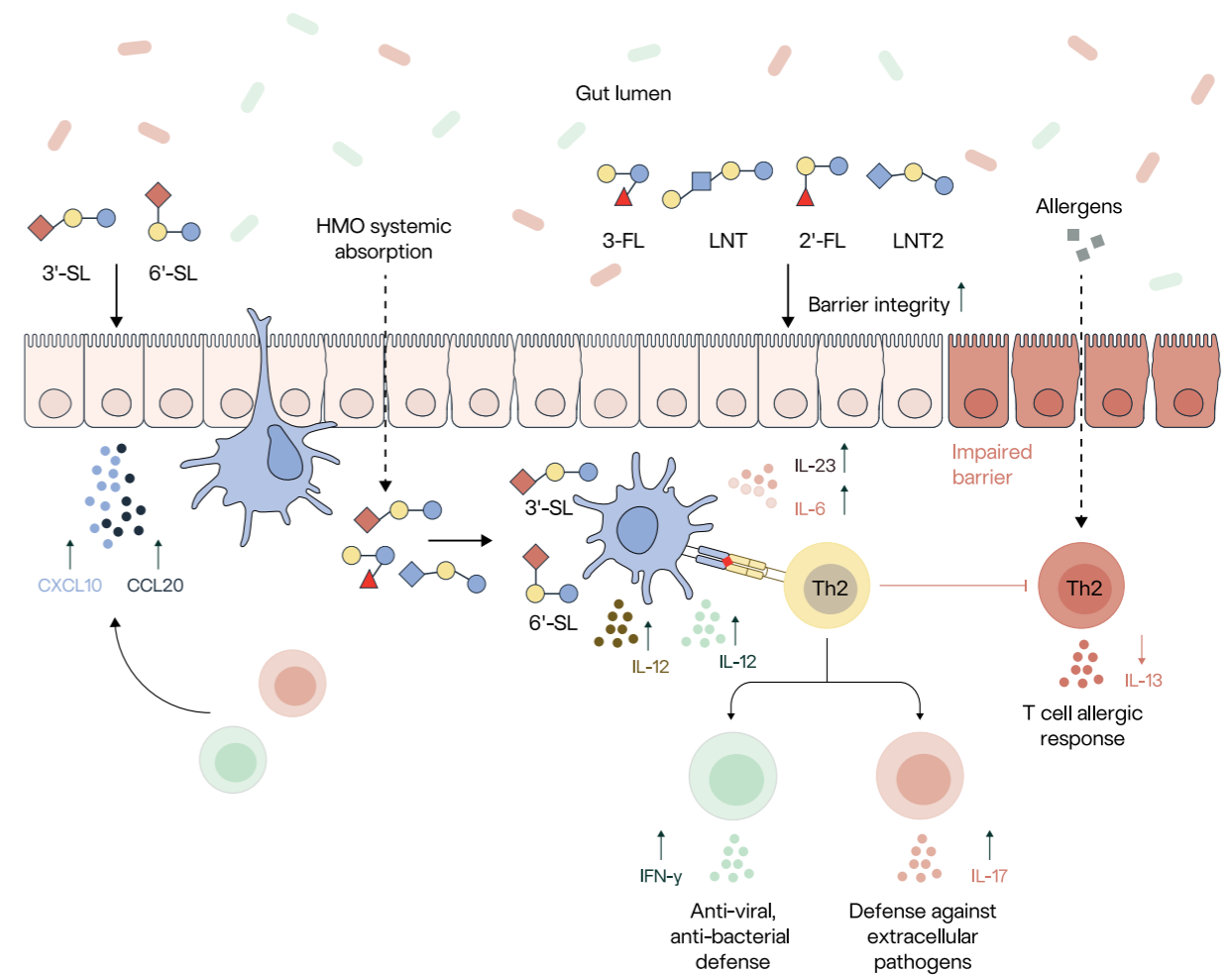
## Executive summary:

In this study, different structured HMO was assessed (2'-FL, 3-FL, LNT, LNT2, 3'-SL and 6'-SL) to investigate the structure-dependent effects of individual HMO on intestinal epithelium, innate and adaptive immune cells. Blood samples were collected from healthy donor for isolating and generating the immune cells. Immune cells were LPS activated and stimulate with or without HMO. Cytokine fingerprint analysis is performed.

It is shown that there are structure-specific effects. 2'-FL, 3-FL, LNT and LNT2 support intestinal barrier integrity, while 3'-SL and 6'-SL are involved in enhancing immune signaling and modulating immune properties.

## *In-vitro* data indicating structure-function effects of HMOs on gut and immune cells

Structure-dependent effects of HMOs on gut barrier, immune signaling, and T cell priming



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Do you want to advance the understanding of HMOs with us?

Learn more about our program and how we can support your efforts - contact us today to get started!



## HMOs for innovation projects

At Novonesis, we are committed to advancing knowledge around HMOs and developing further application opportunities for infant formula and dietary supplements.

Through our program, we facilitate scientific collaboration and offer HMO samples to members of scientific and academic communities. We are also open to potential partnerships.

At Novonesis, we are at the forefront of HMO research innovation. Our donation program plays a crucial role in advancing the development and understanding of Human Milk Oligosaccharides. By fostering collaborations between academia and industry, we are driving progress and unlocking new possibilities in this vital field.

## Follow us



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