RECENTLY PUBLISHED HUMAN MILK COHORT STUDIES

NEW INSIGHTS INTO THE TRANSFORMATION OF HUMAN MILK COMPOSITION DURING THE FIRST YEAR OF LIFE

DANONE NUTRICIA RESEARCH

Aim of human milk cohort studies:

- **Reveal new and reliable insights into changes of human milk composition during first year of life**
- **Application of state-of-the-art instrumental analysis to investigate a sufficiently large number of human milk samples**

> 1000 European (German) human milk samples analyzed

Main collaborators: University of Leipzig, Germany and University of Ulm, Germany (ESPAT human milk cohort and LIMCA human milk cohort)

OBJECTIVES:

Accurate determination of human milk compositional details with regard to contained human milk oligosaccharides (HMOs), lactose, fatty acids (FAs), and amino acids (AAs - free and conjugated).

To reveal or confirm associations of these (individual) human milk compounds (HMOs, Lactose, FAs, AAs) and (beneficial, long term) health effects conveyed to breast fed infants.

In this infographic we will focus on findings related to determination and description of human milk compositional variations.

HUMAN MILK ANALYSIS

Progression of lactation over different stages

Human milk is the best possible nutrition for all infants

The unique composition of human milk provides specific short- and long-term health benefits for the infant such as, amongst others, optimal growth, healthy development[1-3] and protection against infections and diseases.[1-12]

References: 1. WHO 2003 **2.** Jarson, et al. Dev Rev 2008; **3.** Hahn-Holbrook, et al. 2012; **4.** Das, et al. J Affect Disord 2015; **5.** Sivakar, et al. J Perinatol. 2009; **6.** Susman, et al. Am J Psych. 1988; **7.** Kramer MS, et al. The Cochrane Library 2009; **8.** WHO 2008; **9.** Sachdev, et al. Lancet 1991; **10.** WHO 1997; **11.** Agostoni, et al. J Pediatr Gastroenterol Nutr. 2009; **12.** Horta, et al. Food Nutr Res. 2013

LACTOSE & HUMAN MILK OLIGOSACCHARIDES

Carbohydrates represent the largest group of macronutrients in breastmilk, and include lactose and Human Milk Oligosaccharides (HMOs).[13]

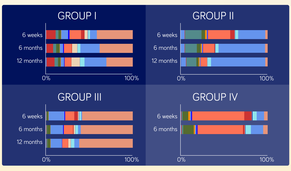
Lactose serves as an energy source, human milk lactose provides around 40% of total energy for the infant.[14]

Human milk oligosaccharides (HMOs) represent the 3rd most abundant fraction of biomolecules found in human milk.[15-17]

- HMOs may support healthy development of the infant by many beneficial effects (prebiotic, anti-infective, and immunomodulatory as well as impact on developing gut (microbiome) etc.).[16,17]
- HMO concentrations are highly variable between mothers and change over the course of lactation.[18]
- Maternal genetics (expression of Secretor and Lewis genes) leads to different HMO profiles in human milk. These specific HMO profiles allow assignment of individual milks to one of 4 different human milk groups.[19,20]

HMO-VARIATIONS IN MILK GROUPS FROM 6 WEEKS TO 12 MONTHS POST PARTUM

The overall distribution of human milk groups was similar as described in literature. 74% was attributed to human milk group I, 18% to human milk group II, 7% to human milk group III, and 1% to human milk group IV. In addition, we were able to distinguish the trajectories of individual HMOs, from 6 weeks post-partum to 12 months post-partum, in the different human milk groups as depicted below.



NEW INSIGHTS INTO LACTOSE & HMOS

Lactose concentrations increased at 6 months and decreased significantly at 12 months compared to 6 weeks of lactation.

Total HMO concentrations decreased significantly at 6 months (β = −1.3487, p < 0.0001) and 12 months (β = −1.0606, p < 0.0001) compared to 6 weeks of lactation, regardless of secretor status or human milk group.

Although the overall content of HMOs decreased (even after correction for early life factors like pre-pregnancy BMI, parity, gestation period, delivery mode, exclusive breastfeeding, and gender of the infant) concentrations of some structures increased (i.e. 3'-SL, 3-FL, DFL).

Some individual HMO isomers (3'-SL & 6'-SL as well as 2'-FL & 3-FL) showed opposite trajectories over the course of lactation: 3'-SL and 3-FL increased, whereas 6'-SL and 2'-FL decreased.

Total combined HMOs (sum of 14 detected structures; mean values) were significantly lower in human milk group IV (5.02 g/L) compared to group I (5.76 g/L) , group II (4.30 g/L), and group III (6.44 g/L).

Individual HMOs independent from maternal Secretor and Lewis genotype varied between milk groups such as 3-FL (highest in group II) and LNT (highest in group IV). Most of the other HMOs also differed significantly by secretor status, except 6'-GL, 6'-SL, and LNFP III.

References: 13. Ballard, O. et al. Pediatr. Clin. North Am. 2013; **14.** Martin, C. R. et al. Nutrients. 2016; **15.** Jantscher-Krenn, E. et al. Minerva Pediatr. 2012; **16.** Boehm, G. et al. J Nutr. 2007; **17.** Ayechu-Muruzabal, V. et al. Front Pediatr. 2018; **18.** Bode-Amor. A.A. et al. Front Microbiol. 2019; **19.** Thurl, S. et al. Br J Nutr. 2010; **20.** Lefebvre, G. et al. Front Nutr. 2020

Online publication: https://www.mdpi.com/2072-6643/13/6/1973/htm

FATTY ACIDS

Lipids are the second largest group of macronutrients in human milk.[21] Amongst other functions lipids serve as an energy source: human milk lipids provide ~50% of total energy needed for growth.[21-23]

Human milk lipids are incorporated into large globules (milk fat globules) surrounded by a complex triple-layer membrane (milk fat globule membrane).[24]

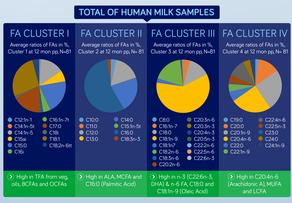
Fatty Acids (FAs) are essential structural elements of human milk lipids (>97% of lipids are FAs). The most abundant FAs in human milk are saturated fatty acids (SFAs), followed by monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs).

- The FA composition in human milk can affect infant growth and (long term) immune, brain and metabolic health.[21-23]
- The FA profile is influenced by maternal fat stores and diet.[21,25]

FA-PROFILES AT 12 MONTH POST PARTUM

Four different FA-clusters emerged after principal component analysis (PCA) of FA profiles as detected in 81 human milk samples collected at 12 months post-partum.
The FA-compounds in the different clusters are highly correlated. However, they vary in chemical properties e.g., degree of saturation, branching, or chain length.

TOTAL OF HUMAN MILK SAMPLES



NEW INSIGHTS INTO FAS

The total lipid content increased over lactation and was significantly higher at 6 months (β = 0.199, p = 0.029) and 12 months of lactation (β = 0.421, p < 0.001) compared to 6 weeks.

Levels of individual FAs are different and vary at 6 weeks, 6 months and 12 months. 4 specific FA-clusters consisting of highly correlated individual FAs could be distinguished by PCA of 45 individual fatty acids from 81 human milk samples. These FA patterns may include compounds with different FA chemical structures (see figure).

MCFAs (medium chain fatty acids) (C12:0 and C14:0) were significantly higher at 6 months & 12 months, vs 6 weeks.

General reduction in n-6 FAs (C20:2n-6, C20:3n-6, C22:2n-6, C18:3n-6) & few n-3 FAs (C20:3n-3 and C20:4n-3) over lactation.

Human milk FA composition (levels) during prolonged lactation is different from that of human milk during a short duration of lactation.

Women's lifestyle changes during prolonged lactation may have contributed to changing human milk FA profiles at 12 months.

References: 21. Koletzko, B. et al. Ann. Nutr. Metab. 2016; **22.** Demmelmair, H. et al. J. Clin. Endocrinol. Metab. 2018; J Clin. Endocrinol. Metab. 2018; **23.** Hageman, JH. et al. Int. Dairy J. 2019; **24.** Gallier, S. et al. Colloids Surf. B. 2015; **25.** Ballard, O. et al. AL. Pediatr. Clin. North Am. 2013

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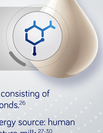
AMINO ACIDS



Human milk contains proteins, these are compounds consisting of amino acids (AAs), which are connected by peptide bonds.[26]

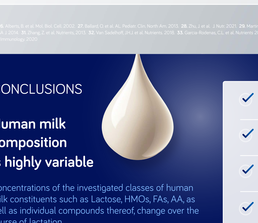
In addition to other functions, proteins serve as an energy source: human milk proteins provide around 7% of total energy in mature milk.[27-30]

Human milk also contains free amino acids (FAAs), which account for 4–10% of the total AA content in human milk.[31,32]

- Infant and maternal characteristics influence the levels of FAAs and total amino acids (TAAs, i.e., the sum of conjugated AAs and FAAs) in human milk.[31,32,33]
- TAAs can be used to estimate the combined protein and peptide levels.[34]
- FAAs can confer immunological benefits such as protection against neonatal allergies or infections.[34]

FREE AND CONJUGATED AA RATIOS AT 6 WEEKS & 6 MONTH POST PARTUM

At 6 weeks 3.7% (a) and at 6 months 5.8% (c) of AAs found in human milk are represented by FAAs. The majority of AAs found in human milk are bound in human milk proteins (>96% TAAs at 6 weeks (a) and >94% TAAs (c) at 6 months). The relative abundance of individual FAAs at 6 weeks and 6 months are visualized in figures b and d, respectively. The two most abundant FAAs in human milk are glutamate and glutamine.



NEW INSIGHTS INTO FA (FREE & CONJUGATED)

The present study showed that the FAAs glutamine and glutamate are highly abundant in human milk, both at 6 weeks (56.3%) and at 6 months (61.5%).

The sum of FAAs was higher at 6 months compared to 6 weeks of lactation. Temporal changes of FAAs in human milk between 6 weeks and 6 months of lactation were AA-specific.

Our results suggest gender related differences in temporal changes of the sum of FAA concentrations (not significant but by trend). Levels of most FAAs were slightly higher in human milk for boys in early lactation, but not at later stages of lactation.

Free glutamine, glutamate, and serine in 6-week human milk positively correlated with infant weight gain in the first 4–5 weeks of life.

These results suggest that specific FAAs may play a role in infant growth.

References: 26. Hinde, K. et al. Mol. Biol. Evol. 2012; **27.** Ballard, O. et al. AL. Pediatr. Clin. North Am. 2013; **28.** Zhu, J. et al. J. Nutr. 2021; **29.** Martin, C.R. et al. Nutrients. 2016; **30.** EFSA. EFSA J. 2009; **31.** Zhang, Z. et al. Nutrients. 2021; **32.** Van Sadelhoff, JH.J. et al. Nutrients. 2018; **33.** Garcia-Rodenas, C.L. et al. Nutrients. 2016; **34.** Van Sadelhoff, JH.J. et al. Front Immunol. Immunology. 2020

Online publication: https://www.mdpi.com/2072-6643/13/6/2009/htm

CONCLUSIONS

Human milk composition is highly variable

Concentrations of the investigated classes of human milk constituents such as Lactose, HMOs, FAs, AA, as well as individual compounds thereof, change over the course of lactation.

These changes may be biologically tailored to specific needs of infants at defined stages of lactation and each compound may have a specific role in supporting healthy infant development.

- Lactose concentrations peaked at 6 months and decreased significantly at 12 months compared to 6 weeks of lactation.
- Individual HMOs such as 3-FL, DFL, and 3-SL increased over progressing lactation.
- We were able to confirm the existence of 4 different human milk groups as distinguishable by their characteristic HMO-profiles.
- 4 different FA clusters emerged after analyzing the total of human milk samples collected at 12 months post-partum.
- While overall HMOs and conjugated AAs declined over the course of lactation, most free AAs and the sum of FAAs increased

OUR EXPERTISE

At Danone Nutricia Research we conduct pioneering research into the composition and benefits of human milk using state-of-the-art analytical equipment. Our research areas focus on (functional) human milk proteins and peptides, oligosaccharides, beneficial bacteria, and the fat architecture of human milk.

50
Years of expertise
Human milk research

Glycobiology
Associate Professorship in Glycobiology linked to Human Milk Research at Utrecht University

300+
Publications
in the area of human milk and breastfeeding

Partnerships
in the field of human milk research

FUTURE RESEARCH FOCUS AREAS

In the future we will continue to closely collaborate with leading birth cohorts studies, apply in-depth analytics of human milk and use advanced biostatistic models.
Stay tuned for further studies intending to investigate the associations between human milk compounds and their beneficial impact on (long term) development of healthy infants.

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