IDENTIFICATION AND CHARACTERIZATION OF CD57-LIKE OLIGOSACCHARIDES IN HUMAN MILK

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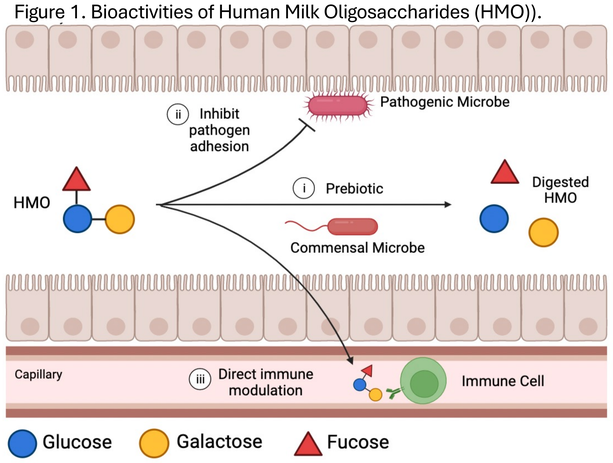
Introduction

Human Milk Oligosaccharides (HMO) are short carbohydrates that constitute breast milk's third most abundant solid component. They confer biological activities that promote infant health and development. HMO serves three major purposes: (i) promote the growth of gastrointestinal microbiota, (ii) inhibit pathogen adhesion, and (iii) modulate the immune system[1].

Figure 1. Bioactivities of Human Milk Oligosaccharides (HMO)).



Genetic factors are a major source of HMO variation. The secretor gene encodes for **α**1-2-fucosyltransferase (FUT2). FUT2-positive (Se+) women produce high concentrations of fucosylated HMOs like 2'FL (see above) while individuals with inactive secretor gene have below-detection concentrations.

Objectives

- To determine if novel sulfated and glucuronic acid-containing HMOs (CD57-like) are present in human milk
- If present, deduce if secretor status influences the relative abundance
- Test the hypothesis that sulfated HMOs do not function as prebiotics.

What is CD57?

The Zandberg Lab discovered a new class of sulfate-containing bovine milk oligosaccharide that attaches to a glucuronic acid (GlcA) moiety[2]. The sulfate and GlcA combination resemble a glycoepitope called CD57, known for interfering with immune cell terminal differentiation and brain activity[3].

Method

1. Past published data set was reanalyzed for novel HMOs containing GlcA residues[4]
2. Extracted milk and feces samples from 4 mother-infant pairs to test the hypothesis in the HMO function
3. All fecal samples were collected from exclusively breastfed infants

Identification by HPLC-MS

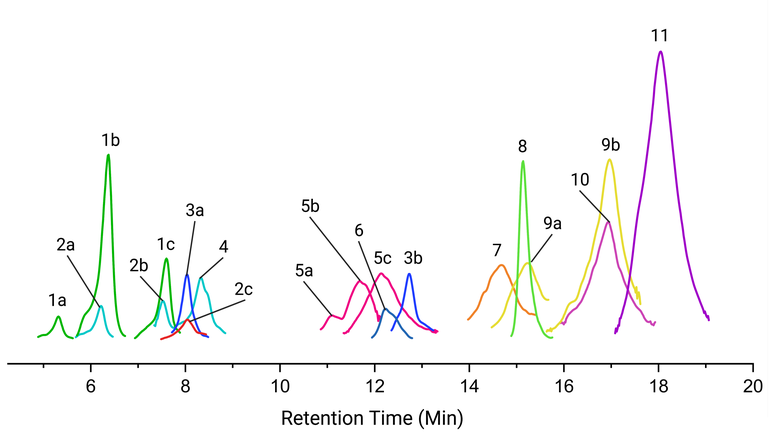
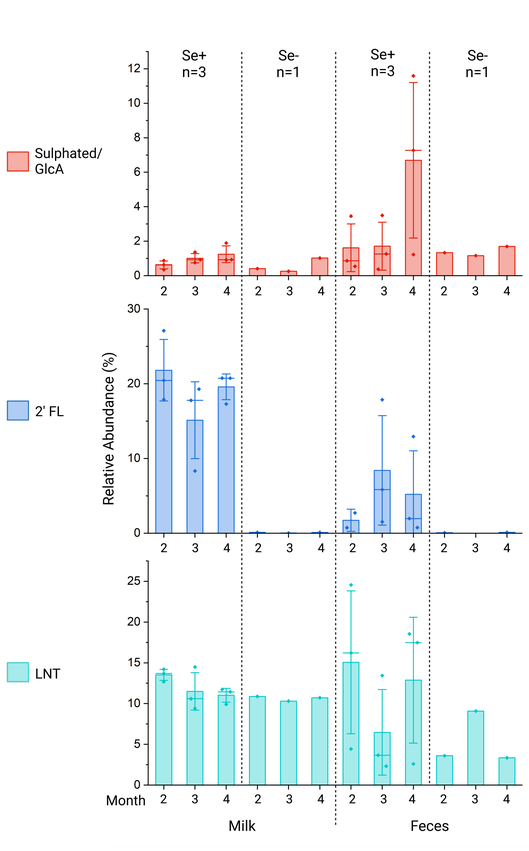


Figure 2. Extracted ion chromatograms (EICs) were generated upon reanalysis of published[4] HPLC-MS data sets from 16 milk samples. The table contains peak information. The code denotes the number of *N*-acetylhexosamine (GlcNAc or GalNAc), hexose (Glc or Gal), fucose, *N*-acetylneuraminic acid (Neu5Ac) and glucuronic acid (GlcA), respectively. S indicates the presence of sulfate and the number after the underscore (_) designates the HPLC-resolved isobar.

Label	Code	Formula	RT	Exact Mass	Error
1a	02101S_1	C24H42O24S	5.318	746.18	1.75
1b	02101S_2	C24H42O24S	6.384	746.1796	1.24
1c	02101S_3	C24H42O24S	7.6	746.1805	2.39
2a	02001S_1	C18H32O20S	6.215	600.1221	2.16
2b	02001S_2	C18H32O20S	7.519	600.122	2.07
2c	02001S_3	C18H32O20S	8.325	600.124	5.37
3a	21001_1	C28H48N2O22	11.698	764.2775	9.95
3b	21001_2	C28H48N2O23	12.739	764.277	9.38
4	11001	C20H35NO17	8.036	561.1861	-7.82
5a	02000S_1	C12H24O14S	11.11	424.0886	-0.22
5b	02000S_2	C12H24O14S	11.693	424.0888	0.25
5c	02000S_3	C12H24O14S	12.543	424.0884	-0.7
6	02001	C18H32O17	12.26	520.164	0.09
7	13100S	C32H57NO28S	14.673	935.2759	-3.07
8	24300S	C58H100N2O46S	15.087	1592.5163	-6.58
9a	24200S_1	C52H90N2O42S	15.239	1446.4646	-2.97
9b	24200S_2	C52H90N2O42S	16.56	1446.4549	-9.68
10	24100S	C46H80N2O38S	16.929	1300.4108	-0.14
11	02100S	C18H34O18S	18.052	570.1458	-1.43

Analysis of breastfed babies' feces to elucidate HMO bioactivity



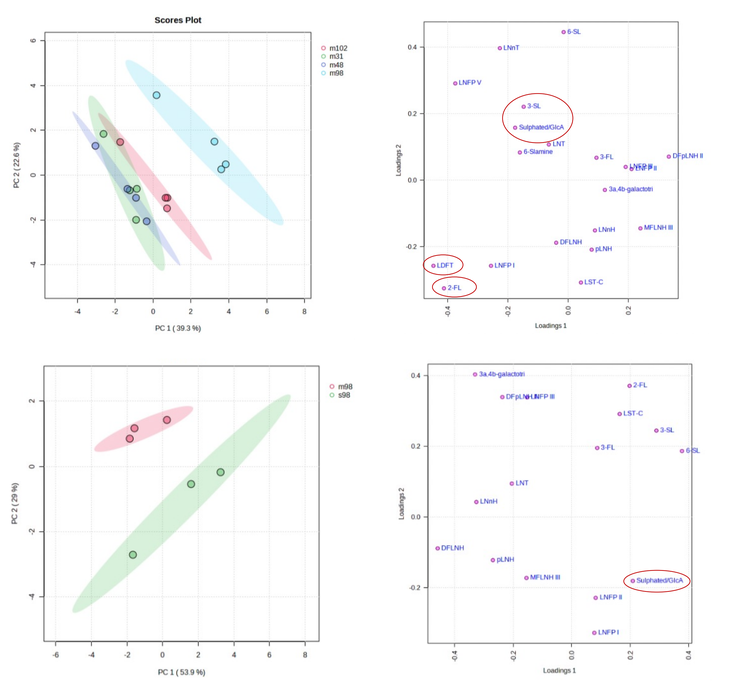


Figure 3. Sulfated/GlcA HMOs are highly enriched in feces collected from breastfed infants. Bar graph exhibit relative abundances (%) of putative sulfated/GlcA HMOs, 2-fucosyllactose (2' FL), and lacto-*N*-tetraose (LNT). Principal Component Analyses (PCA) to evaluate variation within milk samples and differences between milk and fecal samples.

Conclusion

- CD57-like HMOs were discovered in human milk for the first time
- First study to analyze fecal samples from exclusively breastfed infants
- Sulfated and glucuronic-containing HMOs are highly enriched in fecal samples from breastfed infants
- Relative HMO concentrations remained relatively stable during a longitudinal study of 4 months
- Sulfation and sialylation do not appear to compete in humans, as reported previously in bovine samples[2]

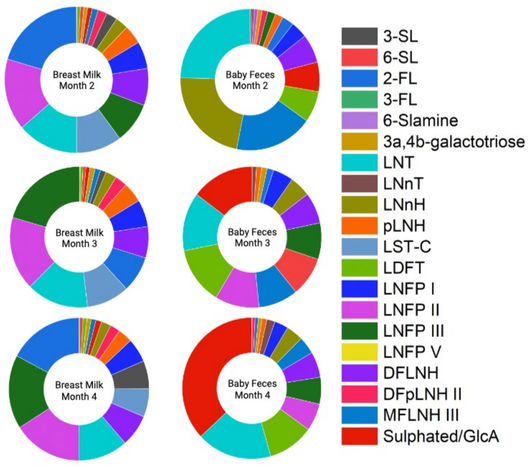


Figure 4. HMO relative abundance (%) compared in milk and feces samples. All except sulfated/GlcA HMOs were identified using standards. The pie charts depict abundances from one Se+ individual.

Acknowledgements

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