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Integrated Analysis of Human Milk Microbiota, Oligosaccharides and Fatty Acids in the Canadian Healthy Infant Longitudinal Development (CHILD) Cohort

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Background:

Human milk contains many bioactive components that have been separately associated with infant development. However, these components are rarely studied in combination.

Objective:

We performed an integrated analysis of three bioactive components of human milk: microbiota, oligosaccharides, and fatty acids.

Methods:

We studied the milk composition of 393 lactating mothers in the CHILD study. Microbiota (n=2255 operational taxonomic units) was analysed by Illumina 16S rRNA gene V4 sequencing. Human milk oligosaccharides (HMOs, n=19) were analysed by HPLC. Milk fatty acids (MFA, n=27) were analysed by gas chromatography. Centre log-ratio transformation and hierarchical clustering were applied to microbiota. Associations between milk components were assessed using multivariable linear regression, redundancy analysis (for overall compositional profiles), and Spearman correlation and network visualization (for individual components). P-values were adjusted for multiple comparisons.

Results:

HMO and MFA profiles were not associated with milk microbiota richness, diversity, taxonomic or functional clusters. In redundancy analyses, HMO profile accounted for 5% of variation in the microbiota composition ($p = 0.042$) while MFA profile accounted for 8% of variation in the microbiota ($p=0.033$). HMO and MFA profiles significantly accounted for 20% and 11% of variation in MFA and HMO, respectively. In assessing members of each component individually, we identified several significant associations including DSLNT HMO as well as C18:1n9, C24:0, and C24:1n9 fatty acids with microbiota composition. Firmicutes, Proteobacteria and *Veillonellaceae* were significantly correlated with the HMO DFLac. Multiple significant yet weak correlations were identified between microbiota and individual MFAs including Fusobacteria with C12:0, t18:1n7, C22:5n3, and C16:1n9, and Firmicutes and Proteobacteria with C16:1n9 and C20:4n6. Overall, correlations within each component were stronger than between different components.

Conclusion:

Using multiple approaches to integrate and analyse milk microbiota, HMO, and MFA, we observed several novel associations. Additional research is needed to characterize these associations and determine their clinical significance.