Expression of Inflammation Associated Genes in Extracellular RNA of Mammary Origin in Lactating Women According to Plasma Triglyceride Status

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Introduction: The benefits of breastfeeding are unparalleled; unfortunately, the obesity and diabetes epidemics are fueling a dramatic rise in the number of mothers who are physiologically unable to produce sufficient breast milk. Here, we examine the expression of inflammation-associated genes in extracellular RNA derived from the lipid fraction of breast milk to identify mammary epithelial cell (**MEC**) expression signatures during lactation that characterize insufficient breast milk production in women with obesity and elevated plasma triglycerides.

Hypothesis: Genes involved in inflammatory signaling pathways in the MEC of lactating mothers will be differentially expressed in women with obesity and hypertriglyceridemia compared to those with normal plasma triglyceride status.

Methods: Fresh breastmilk samples were collected from 10 mothers with low milk production (0-254 mL/24h, **LOW**), and 8 control mothers with sufficient milk production (562-801 mL/24h, **CON**). All LOW mothers were obese and had significantly elevated plasma triglycerides as compared to CON mothers. The lipid fraction of freshly expressed human milk is a rich source of extracellular RNA of MEC origin. We isolated total RNA of this source and conducted RNA-sequencing. From the resulting transcriptome, we intersected differentially expressed genes with KEGG inflammatory signaling pathways (cytokine-cytokine interactions, NF-κβ signaling, and Toll-like Receptor signaling).

Results: Of 402 KEGG inflammatory pathway genes, 211 are expressed in the lactating MEC. Thirty-three of the 211 were differentially expressed ($P<0.05$), with 27 that were upregulated by ≥1.2-fold and 6 that were downregulated by ≤0.7-fold. Differential expression included enrichment of proinflammatory genes (CXCL17, CX3CL1, CXCL16, CCL28, IL34, IL10RB, HVEM and FN14) and downregulation of anti-inflammatory genes (CSF1 and OPG).

Conclusions: Analysis of the MEC transcriptome provides a magnified snapshot of the delicate interplay of cellular networks, inflammatory signaling and immunoregulation. Upregulation of FN14 (which is implicated in the induction of apoptosis), and downregulation of OPG (known to protect against apoptosis), in association with upregulation of genes encoding chemokine proteins and their receptors, may compromise the viability of MEC during lactation. Further elucidation of the effects of obesity-induced inflammation on MEC function during lactation may enable the development of targeted interventions to improve human milk quantity and quality.

Acknowledgements: This study was funded by NIH grant T35DK060444 through the Medical Student Summer Research Program (MSSRP) at the University of Cincinnati College of Medicine; NIH 5 K12 HD051953 (PI, Tsevat) Bridging Interdisciplinary Research Careers in Women's Health (BIRCWH award to LN-R); NIH UL1 TR001425; and NIH P30 DK078392 (bioinformatics core) of the Digestive Diseases Research Center**.** The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.