TABLE OF CONTENTS

PUBLICATIONS AND SCIENTIFIC CONTRIBUTIONS

Included papers

Paper I *(Appendix I)*

Christensen SH, Lewis JI, Larnkjær A, Frøkiær H, Allen LH, Mølgaard C, Michaelsen KF (2022): Associations between maternal adiposity and appetite-regulating hormones in human milk are mediated through maternal circulating concentrations and might affect infant outcomes. *Front Nutr. 2022 Nov 4;9:1025439. doi: 10.3389/fnut.2022.1025439*

Paper II *(Appendix II)*

Christensen SH, Rom AL, Greve T, Lewis J, Frøkiær H, Allen L, Mølgaard C, Renault KM, Michaelsen KF (2023): Maternal inflammatory, lipid and metabolic markers and associations with pregnancy and breastfeeding outcomes. *Under review, Frontiers in Nutrition, June 2023.*

Paper III *(Appendix III)*

Christensen SH, Lewis JI, Frøkiær H, Johnsen PR, Peerson J, Tan X, Shahab-Ferdows S, Hampel D, Islam M, Kac G, Mucci DB, Figueiredo ACC, Moore S, Mølgaard C, Allen LH, Michaelsen KF (2023): Cytokines and appetite-regulating hormones in human milk and associations with infant growth across four sites: The Mothers, Infants and Lactations Quality study. *Ready for submission, June 2023.*

OTHER SCIENTIFIC CONTRIBUTIONS

Authorships

Larnkjær A, Larsson MW, Wells J, **Christensen SH**, Lewis JI, Mølgaard C and Michaelsen KF: Cohort with excessive weight gain during exclusive breastfeeding – follow-up at 36 months of age. *In preparation for submission to Nutrients.*

Larnkjær A, Lewis JI, **Christensen SH**, Mølgaard C and Michaelsen KF: Early Nutrition and Its Effect on Growth, Body Composition, and Later Obesity. *World Rev Nutr Diet. 2023;126:140-155. doi: 10.1159/000527948*

Lewis JI, **Christensen SH**, Larnkjær A, Mølgaard C, Michaelsen KF: Early Nutrition and Its Effect on Growth, Body Composition, and Later Obesity. *World Rev Nutr Diet. 2022;125:138-155. doi: 10.1159/000521798*

Allen LH, Hampel D, Shahab-Ferdows S, Andersson M, Barros E, Doel AM, Eriksen K, **Christensen SH**, Islam M, Kac G, Keya DK, Michaelsen KF, Mucci DB, Njie F, Peerson JM and Moore SE: The Mothers, Infants, and Lactation Quality (MILQ) Study: A Multi-Center Collaboration. *Curr Dev Nutr 2021 Sep 20;5(10):nzab116. doi: 10.1093/cdn/nzab116*

Larnkjær A, **Christensen SH**, Lind MV, Michaelsen KF and Mølgaard C: Plasma vitamin B12 concentration is positively associated with cognitive development in healthy Danish 3-year-old children: the SKOT cohort studies. *Br J Nutr. 2022 Nov 28;128(10):1946-1954. doi: 10.1017/S0007114521004888*

Christensen SH, Grenov B, Larnkjær A, Mølgaard C and Michaelsen KF: Early Nutrition and Its Effect on Growth, Body Composition, and Later Obesity. *World Rev Nutr Diet. 2021;123:122-135. doi: 10.1159/000516440*

Eriksen KG, **Christensen SH**, Lind MV and Michaelsen KF: Human milk composition and infant growth. *Curr Opin Clin Nutr Metab Care. 2018 May;21(3):200-206. doi: 10.1097/MCO.0000000000000466.*

Larsson MW, Larnkjær A, **Christensen SH**, Mølgaard C and Michaelsen KF: Very high weight gain during exclusive breastfeeding followed by slowdown during complementary feeding: Two case reports. *J Hum Lact. 2019 Feb;35(1):44-48. doi: 10.1177/0890334418756580.*

Conference presentations

Oral presentation at the Nutrition & Growth conference (England, March 2023): Maternal inflammation and metabolic markers during pregnancy and associations with birth-related and breastfeeding outcomes.

Oral presentation at the ISRHML conference (Panama, October 2022): Associations between maternal body composition and human milk appetite-regulating hormones are partly mediated through maternal circulating hormones.

Oral presentation of the Yearbook chapter for Nutrition & Growth conference (Virtual, August 2021): Early Nutrition and Its Effect on Growth, Body Composition and Later Obesity.

Poster presentation at the ISRHML conference (Virtual, August 2021): Higher human milk fat and protein are associated with lower milk intake in Danish infants from the MILQ cohort.

Poster presentation at the ISRHML conference (Japan, October 2018): Lipidomics of human milk and plasma from exclusively breastfed infants with excessive weight gain.

ABSTRACT

Background: Overweight and obesity among children is an increasing concern, partly due to the substantial economic burden for the public health care system as childhood obesity increases the risk of obesity in adulthood with a subsequent risk of type 2 diabetes and cardiovascular diseases. With an increasing prevalence of the double burden of malnutrition, where under- and over nutrition coexist, overweight and obesity persist a major concern in a global perspective. The mechanisms whereby overweight and obesity are passed onto generations include foetal and lactational programming. Through inflammatory conditions as well as alterations of glucose and lipid metabolism during pregnancy, offspring of mothers with obesity may be predisposed to an altered appetite regulation as well as lipid and glucose metabolism. In early infancy, when physiological mechanisms may not be fully developed, orally ingested bioactives from human milk (HM) are posed to affect infant appetite regulation and possibly infant growth. Studies indicate that HM of mothers with compared to without obesity have elevated concentrations of the hormones leptin and insulin as well as cytokines including Tumour-necrosis factor-α and interleukin-6. As such, maternal health status may affect the infant during pregnancy and possibly lactation. However, the evidence for these mechanisms is strongest among women with obesity or with diseases such as gestational diabetes mellitus, and less is known about the mechanisms in mothers with normal- or overweight and without disease.

Objectives: The main objective was to explore the interplay between biomarkers of maternal inflammation during pregnancy and lactation and infant outcomes within a healthy population. Secondly, maternal predictors of inflammation during pregnancy and lactation were investigated.

Method: Data from Mothers, Infants and Lactation Quality (MILQ) was used in the present PhD thesis. The MILQ study is a cohort study including 1,000 healthy mother-infant dyads from Bangladesh, Brazil, Denmark and The Gambia with data collected at three time points from 1 to 8.5 months after birth. In **Paper I**, data from Denmark were used to investigate the mediating pathway by which appetite-regulating hormones (ARHs) arrive in HM. Data from a subgroup in Denmark were used in **Paper II** to explore associations between inflammatory-, lipid- and metabolic markers in pregnancy and pregnancy and breastfeeding outcomes. Data from all four cohorts were used in **Paper III** to compare HM concentrations of cytokines and ARHs between the sites as well as investigate the associations with infant growth.

Results: In **Paper I**, we found that leptin concentrations in HM were higher for mothers with body mass index (BMI) ≥25 kg/m^2 compared to <25 kg/m^2 with a higher mediated effect through plasma levels for mothers with BMI ≥25 kg/m^2. A mediated effect was found in the association between maternal fat mass index and HM insulin, while no association was found neither for HM adiponectin nor between HM ARHs and infant outcomes. The findings support that leptin and insulin arrive in HM through maternal plasma, and to a higher degree for mothers with overweight.

In **Paper II**, higher levels of triglycerides and glucose following an oral glucose tolerance test were associated with higher birthweight adjusted for gestational age (GA), whereas higher levels of high-density lipoprotein were associated with a lower GA-adjusted birthweight and shorter duration of pregnancy and exclusive breastfeeding (EBF). Metabolic markers related to insulin resistance were positively associated with duration of EBF. Finally, higher pre-pregnancy BMI was associated with increased inflammatory and metabolic markers. The results indicate that lipid and glucose metabolism during pregnancy might affect foetal growth possibly through the placenta. Metabolic markers may affect duration of EBF, although these results were possibly confounded by mothers receiving breastfeeding counselling.

In **Paper III**, concentrations of cytokines and ARHs in HM differed significantly among the four sites of the MILQ study. Cytokines related to a T-helper cell type 2 (Th2) dominated immune response were highest in HM from Denmark, while concentrations of the three ARHs were highest in Brazil and lowest in Bangladesh. Furthermore, most cytokines in HM were inversely associated with weight-for-age and weight-for-length Z-scores in The Gambia. Our results indicate that cytokines in HM may reflect different environmental exposures, which possibly explain declining Z-scores identified in The Gambia.

The evidence of associations within HM research is sparse as the observational nature of most research studies often results in a substantial risk of confounding. However, results from the present PhD thesis indicate that maternal weight status may affect inflammatory biomarkers and metabolic alterations during pregnancy while also affecting HM composition of cytokines and ARHs. Furthermore, the alterations during pregnancy may affect foetal growth through the placenta. Finally, different environmental exposures are likely reflected in HM composition of cytokines, which possibly explain infant growth in certain populations. Breastfeeding remains an essential health promoting element in a global perspective, although more studies are needed to confirm the mechanisms and the influence on infant outcomes in healthy populations.

RESUMÉ (Danish abstract)

Baggrund: Stigningen i forekomsten af overvægt og svær overvægt er en stor økonomisk byrde for særligt den offentlige sundhedssektor da overvægt i barndommen øger risikoen for overvægt senere i livet samt overvægtsrelaterede sygdomme såsom type 2 diabetes og hjertekarsygdomme. Med stigende prævalens af *double burde of malnutrition*, hvor både under- og overernæring forekommer samtidig, i lavindkomstlande, er overvægt en stor udfordring i et globalt perspektiv. Videreførelsen af overvægt forekommer blandt andet via føtal og ernæringsrelateret programmering. Mekanismer som involverer et ændret inflammatorisk respons samt en ændret lipid- og glukose metabolisme hos moderen, medfører at børn af mødre med svær overvægt er disponeret for en ændret appetitregulering samt tilsvarende ændret glukose metabolisme. Efter fødslen, hvor fysiologiske mekanismer ikke er fuldt udviklede, er bioaktive komponenter i modermælk foreslået at kunne påvirke appetitregulering og muligvis vækst. Studier indikerer at mødre med svær overvægt har øgede koncentrationer af hormonerne leptin og insulin samt inflammationsmarkørerne Tumour-necrosis factor-α og interleukin-6 i modermælken. Moderens sundhedstilstand kan derved påvirke barnet både under graviditeten samt via modermælken efter fødslen. Evidensen for de ovennævnte mekanismer er størst blandt mødre med svær overvægt eller sygdomme såsom gestationel diabetes. Vi ved dog mindre om de mekanismer der regulerer børns vækst under graviditet og amning blandt mødre med normal- eller overvægt og uden sygdomme.

Formål: Hovedformålet med denne PhD var at undersøge samspillet mellem biomarkører for inflammation samt appetit-regulerende hormoner i graviditeten samt i modermælk og udfald for barnet. Herudover blev prædiktorer for disse biomarkører i modermælk undersøgt.

Metode: Data fra Mothers, Infants and Lactation Quality (MILQ) blev anvendt i denne PhD. MILQ er et kohorte studie med 1000 raske mor-barn par inkluderet fra henholdsvis Bangladesh, Brasilien, Danmark og Gambia med data indsamlet ved tre tidspunkter mellem 1 og 8,5 måned efter fødslen. I **Artikel I** blev data udelukkende fra Danmark anvendt til at undersøge den medierede effekt i sammenhænge mellem moderens kropssammensætning og appetit-regulerende hormoner i modermælk. Data fra en subgruppe i Danmark blev brugt i **Artikel II** til at undersøge biomarkører for inflammation samt lipid- og glukose metabolisme i graviditeten og udfald for barnet. Data fra alle fire lande blev anvendt i **Artikel III** til at sammenligne koncentrationer af cytokiner og hormoner i modermælk samt undersøge sammenhænge med vækst.

Resultater: Resultater fra **Artikel I** viste, at leptin koncentrationer i modermælk var højere fra mødre med BMI ≥25 kg/m^2 sammenlignet med <25 kg/m^2 samt at den medierede effekt gennem plasmakoncentrationer var højere blandt mødre med BMI ≥25 kg/m^2. En medieret effekt gennem plasma blev ligeledes fundet mellem moderens fedtmasse indeks og insulin i modermælk. Vi fandt ingen sammenhæng med adiponektin i modermælken eller mellem hormoner i modermælk og udfald hos barnet. Resultaterne indikerer at leptin og insulin i modermælk er delvist medieret gennem plasmakoncentrationer og potentielt i højere grad for mødre med overvægt.

I **Artikel II** så vi, at øgede triglycerid- og glukosekoncentrationer efter en oral glukose belastningstest var associerede med en højere fødselsvægt justeret for gestationsalder (GA), hvorimod højere niveau af højdensitetslipoproteinkolesterol var associeret med lavere GA-justeret fødselsvægt samt kortere varighed af graviditet og eksklusiv amning. Metaboliske markører var associeret med en længere varighed af eksklusiv amning og højere BMI før graviditeten var relateret til øgede koncentrationer af biomarkører for inflammation samt metabolisk funktion i graviditeten. Resultaterne indikerer at moderens lipid- og glukose metabolisme kan påvirke føtal vækst, muligvis via placenta. Metaboliske markører kan potentielt påvirke varighed af amning, omend disse resultater formentlig var påvirkede af at kvinderne modtog ammerådgivning.

I **Artikel III** fandt vi signifikante forskelle i koncentrationerne af cytokiner og appetitregulerende hormoner i modermælken fra de fire lande. Cytokiner relateret til et T-celle type 2 drevet immunrespons var dominerende i mælken fra danske mødre, hvorimod koncentrationer af de tre hormoner var højest i Brasilien og lavest i Bangladesh. Øgede koncentrationer af cytokiner var desuden relateret til lavere vægt-for-alder samt vægt-for-længde Z-scorer i Gambia. Resultaterne indikerer at cytokin-profilen i modermælk afspejler miljømæssige faktorer, som potentielt også kan have en betydning for faldende vækst identificeret i Gambia.

Evidensen inden for forskning omkring graviditet og/eller amning er typisk begrænset af en betydelig risiko for confounding. Resultaterne i denne PhD tyder dog på at raske mødres vægtstatus kan påvirke ændringer i lipid- og glukose metabolisme under graviditeten, hvilket potentielt kan påvirke føtal vækst. Yderligere tyder resultaterne på at både vægtstatus samt miljømæssige faktorer formentlig bidrager til kompositionen af modermælk i raske mødre. Amning forbliver således en essentiel faktor for sundhedsfremme i et internationalt perspektiv, om end flere studier er nødvendige for at fastlægge de specifikke mekanismer bag amning samt amningens betydning for udfald hos barnet i en rask population.