Summary

Background: There is convincing evidence from several meta-analyses that a high weight gain during the first 6 months of life is associated with an increased risk of overweight, obesity and metabolic complications later in childhood and adulthood. Compared to formula fed infants, breastfed infants have an overall slower growth. However, breastfed infants have a more rapid growth in the first 1-2 months, but then it becomes slower for the rest of the first year. Nevertheless, some exclusively breastfed infants experience excessive weight gain during the first 6 months of life. Breastfeeding has been suggested to reduce the risk of later overweight and obesity, but not all studies find a protective effect. Human milk composition varies between women and there is emerging evidence that different components have an effect on growth. Only few studies have investigated infants with excessive weight gain. Two case-reports examined exclusively breastfed infants with excessive weight gain and suggested high protein content in human milk as a potential cause. Both cases experienced a slowdown in weight gain velocity when well into the complementary period. A cohort study examined 65 infants with excessive weight gain during the first year. However, the growth velocity in this cohort continued to be high, also after introduction to complementary foods. Since the literature on excessive weight gain in exclusively breastfed infants is sparse, health personnel lack evidence on which to base advice to parents of these infants.

Objective: The main objectives of this PhD thesis were to explore factors which could have a role in the excessive weight gain observed in some breastfed infants, to describe the growth and body composition pattern of these infants and to discuss potential consequences.

Design and methods: Paper I reports two cases of infants with excessive weight gain during exclusive breastfeeding with information on 24h human milk intake and macronutrient concentration measured at 5.5 (Case 1) and 4 months (Case 2). **Paper II-III** are based on a cohort study, SKOT-III, which includes two groups of exclusively breastfed infants examined from January 2016 to October 2017. Eligible infants for the high weight-gain (HW) group were exclusively breastfed infants with excessive weight gain of at least +1 increment in weight-for-age z-scores (WAZ) during the first 5-6 months, and at 5-6 months a WAZ > 2.0 or a BMI-for-age z-score (BAZ) > 2.0 according to the WHO Growth Standards. Infants with a WAZ at age 4-6 months between -1.0 and +1.0 were qualified for the normal weight-gain

(NW) group. Anthropometry, body composition, milk and blood samples, and milk intake were measured at 5 and 9 months in both groups, and in the HW group anthropometry and body composition were also measured at 18 months. Human milk samples were analysed for macronutrients, energy, hormones, specific proteins, and oligosaccharides, and blood samples were analysed for appetite- and growth hormones and metabolic parameters. Information on breastfeeding characteristics, infant eating behaviour, and family history were obtained by interviews of the parents.

Results: In **Paper I**, we reported two exclusively breastfed infants with an increase in WAZ until they were well into complementary feeding (7 and 9 months, respectively) after which both cases had a rapid decline in WAZ. Case 1 weighed 13.0 kg at 7 months (WAZ +4.2) and Case 2 weighed 18.4 kg at 9 months (WAZ +7.5). The 24 hour milk intake was high, 1132 and 1421 grams, respectively. Fat and lactose concentration in their milk were within normal range for both mothers, whereas protein concentration in the milk was below reference values. In **Paper II**, 13 infants were included in the HW group with a mean WAZ of +3.02 at the 5 months visit and 17 infants were included in the NW group with a mean WAZ of +0.39 at the 5 months visit. All anthropometric measures and body composition were significantly different between the groups at the 5 months visit. The HW group had a mean weight gain of 6.6 kg and a mean WAZ increase of 1.71 from birth to 5 months of age, compared to 4.4 kg and 0.15 unit increase in the NW group. The HW group had 70% more fat mass at 5 months than the NW group. After introduction to complementary foods, weight velocity decreased in the HW group and they had a marked catch-down in WAZ of 1.1 units from 5 to 18 months. Human milk intake and breastfeeding patterns at 5-6 months did not differ between the groups. Milk leptin was lower in the HW group at 5 months and serum leptin was considerably higher at 5 and 9 months compared to the NW group. In **Paper III** we found differences in several human milk oligosaccharides (HMOs) between the HW and the NW group at 5 and 9 months. Lacto-N-neotetraose (LNnT) was low in the HW group compared to the NW group at 5 months, and further at 9 months. When combining the two groups total HMO concentrations, total HMO-bound fucose, and 2´-fucosyllactose at 5 months were positively associated with both fat mass index and weight velocity 0-5 months. LNnT was negatively associated with length-for-age z-score, weight velocity 0-5 months, and fat mass index.

Discussion and conclusion: All infants in the case study and the SKOT-III cohort had marked catch-up growth during exclusive breastfeeding compared to the NW group and WHO growth standards. Further, they all showed catch-down growth when complementary foods were introduced and the majority of the infants reached the normal range of WAZ and BAZ the following year, which suggest that the excessive weight gain is caused by human milk composition and intake. Low milk leptin in the HW group may have stimulated appetite and milk intake when weight gain was high. Unfortunately, human milk intake was measured when most infants were introduced to complementary foods and when weight velocity was decreasing, and although the intake was 130 g higher in the HW group, the difference was not significant. High serum leptin in the HW group is likely to reflect the high body fat mass, and since energy intake was likely to be high, the infants were likely to be leptin resistant which could impact cerebral regulation of energy balance, but this is speculative. Our findings of lower values of the HMO LNnT in the HW group compared to the NW group, and negative association of LNnT with fat mass index is in line with a previously published study finding negative association of LNnT with percentage fat mass. The differences in HMO pattern between the two groups and the associations with weight gain and body composition suggest that the HMO composition could play a role in the excessive weight gain. There were no differences in macronutrient concentration in human milk between the HW group and the NW group, which contradicts the suggestions in the two previously published case-reports, that a high protein concentration was a possible explanation for the high weight gain. Unfortunately we did not measure human milk intake, composition and blood parameters during the period with excessive catch-up growth, which would probably have given us a better understanding of why these infants had this special growth pattern. There is a need to confirm the findings from this thesis in larger studies with longer follow-up. Even though recent data from a large Danish cohort show that the majority of infants with high WAZ at 5 months are not becoming overweight or obese later in childhood, the evidence for the association of early rapid weight gain and later obesity is strong. Therefore, it is important for health personnel to be aware of a possible increased risk of later obesity. We do not suggest intervening in the breastfeeding pattern of infants with excessive weight gain during exclusive breastfeeding, but we find it important to emphasize that the growth of these infants should be monitored closely and when complementary feeding is introduced it is according to current recommendations.