

1 **The influence of maternal obesity and breastfeeding on infant appetite- and growth-related hormone**
2 **concentrations: the SKOT cohort studies**

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12 Running title: Maternal obesity and hormone levels in offspring in late infancy

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22 Key words: pre-pregnancy obesity, infancy, insulin, IGF-I, appetite hormones,

1 **Abstract**

2 *Background/Aims:* Exposure to obesity during pregnancy may lead to adverse changes in the offspring's
3 metabolic profile. We compared appetite- and growth-related hormones in a cohort of infants born to
4 obese mothers (SKOT-II) with infants born mainly to non-obese mothers (SKOT-I).

5 *Methods:* Infants from SKOT-I (n=273) and SKOT-II (n=132) were examined including anthropometric
6 measurements and blood samples analyzed for glucose, insulin, insulin-like-growth factor (IGF-I),
7 adiponectin and leptin. Information on breastfeeding and parental characteristics was also collected.

8 *Results:* At 9-months of age SKOT-II infants were 3.6% heavier and 1.2% longer than SKOT-I infants even
9 though their mothers were shorter. There was no difference in BMI. SKOT-II infants had higher levels of
10 insulin, adiponectin and leptin but lower levels of IGF-I compared to SKOT-I (all $p \leq 0.015$). These differences
11 remained except for leptin when adjusted for current weight. Breastfeeding versus non-breastfeeding at 9
12 months was associated with lower concentrations of all hormones (all $p \leq 0.003$). In adjusted models,
13 maternal BMI at 9 months was positively associated with insulin and adiponectin and negatively with IGF-I.

14 *Conclusions:* Pre-pregnancy obesity confers symmetrically larger infant body size and higher levels of most
15 growth and appetite-related hormones but surprisingly lower levels of IGF-I, suggesting other possible
16 infant growth-promoting effects through insulin.

17

1 Introduction

2 Many studies have reported an association between pre-pregnancy obesity and increased risk of cardio-
3 metabolic risk markers and obesity in the offspring both during childhood and later in life [1-5].

4 To develop effective prevention strategies against obesity it is important to understand the mechanisms
5 involved. Exposure to over-nutrition *in utero* if the pregnant mother is obese and/or have high gestational
6 weight gain (GWG) may alter hormones in the offspring involved in regulating post-natal appetite, growth
7 and adiposity such as adiponectin, leptin, insulin and insulin-like-growth factor (IGF-I) [6-8].

8 Insulin and IGF-I promote growth in infancy and high concentrations may lead to relatively rapid growth
9 which has been associated with later overweight and obesity [9-12]. The IGF system is affected by obesity
10 but conflicting impacts have been observed [13;14]. Thus reduced levels of IGF-I which seems to be
11 reversible upon weight loss [13], borderline lower levels in obese prepubertal obese children [15] as well
12 as increased levels observed in obese children [16] have been reported. However, the influence of being
13 born to obese mother on the IGF-I system in infancy is not widely investigated. Leptin, primarily produced
14 by adipose tissue but also the placenta [17;18], signals satiety and is involved in regulation of energy
15 expenditure and body weight [19]. Adiponectin promotes insulin sensitivity by assisting uptake and
16 metabolism of carbohydrates and fatty acids [20;21]. The role of the appetite-related hormones
17 adiponectin and leptin during fetal development and later in infancy is less clear and only described
18 sparsely, but they may be involved in the central regulation of food intake and energy balance via receptors
19 in hypothalamus and thereby affect growth [8;22].

20 Further to the intrauterine environment, postnatal nutrition may be a modulating factor. Breastfeeding has
21 in many studies been associated with a reduced risk of later obesity [1;23;24]. Breastfed infants show an
22 overall slower growth velocity after the first 2-3 months, compared to formula fed infants and the lower
23 protein content has been suggested as one of the main reasons [25-27]. In accordance with this the
24 hormone levels also seems to be affected by the infant feeding mode and studies have reported different
25 levels of appetite and growth -related hormones in breastfed and formula fed infants but some with
26 conflicting results [28-32].

27 Obese mothers often tend to breastfeed less than normal weight mothers and the effect of breastfeeding
28 on hormone levels may therefore be less among infants of obese mothers [20]. The impact of maternal
29 BMI, infant feeding mode and perinatal factors and the complex interplay of these factors on appetite and
30 growth-related biomarkers in late infancy have only been sparsely investigated [22;33;34] and to our
31 knowledge not all biomarkers in the same cohort .

32 In this study we investigate hormone levels related to appetite and growth using the data from the two
33 Danish cohorts SKOT-I [29] and SKOT-II [35] consisting of children born by mainly non-obese mothers and
34 by obese mothers, respectively. The aim of present paper is to compare the hormone levels of the infants
35 at 9 months of age from the two cohorts and by combining the cohorts we explore the impact of
36 breastfeeding, maternal BMI and other maternal and antenatal factors on the hormone levels in late
37 infancy.

38

1 **Subjects and Methods**

2 **Study design and participants**

3 The study sample was drawn from the two prospective observational cohorts; SKOT-I and SKOT-II mainly
4 differing in the pre-pregnancy BMI of the mothers but using similar protocols for data-collection making it
5 possible to combine the cohorts [35]. Both cohorts have been described previously [29;35]. Briefly, in SKOT-
6 I the inclusion criteria were as follows: healthy, singleton term infants with an age of 9 months \pm 2 weeks.
7 They were recruited from the Copenhagen area by random selection of infants from the National Danish
8 Civil Registry from 2007-2008. For SKOT-II, the mothers had participated in the TOP-study [36] (Treatment
9 of Obese Pregnant Woman at Hvidovre Hospital in the Copenhagen area) i.e. they had a pre-pregnancy BMI
10 $>30\text{kg/m}^2$, and their infants fulfilled the same inclusion criteria as the SKOT-I cohort. The 9 months
11 examination took place from 2011-2012. Both studies were approved by The Committees on Biomedical
12 Research Ethics for the Capital Region of Denmark (SKOT-I: H-KF-2007-0003 and SKOT-II: H-3-2010-122).

13 **Anthropometric measurements**

14 Birth weight and length were obtained from health records. The 9 months examination was conducted at
15 the Department of Nutrition, Exercise and Sports, University of Copenhagen as described in detail
16 elsewhere [28;29;35]. Except for weight the measures were performed in triplicates using the average of
17 the three measurements in the analyses. BMI was calculated as $\text{weight}/\text{length}^2$ (kg/m^2). To adjust for the
18 age at the examination and gender differences the z-scores for weight, length and BMI were calculated
19 using WHO growth standards as reference and the WHO Anthro2005 program [37].

20 **Blood samples**

21 Venous blood samples of 5 ml were drawn as described elsewhere [29;38]. Briefly the infants were fasted
22 up to about 2 h before sampling. Time and content of the last meal was recorded and analyzed using
23 Dankost (version 3000, Dankost Ltd., Copenhagen, Denmark) for later adjustments.

24
25 It was not possible to obtain blood samples from all the infants. Of the 311 and 166 infants who completed
26 the examination in SKOT-I and SKOT-II, respectively, blood samples were obtained from 279 in SKOT-I and
27 133 in SKOT-II. As adiponectin was of one of the hormones of major interest only infants with valid
28 adiponectin measurement were included in the present study corresponding in a total sample size of 406.
29 For analyses of insulin, IGF-I, IGF binding protein-3 (IGFBP-3) and leptin some samples were missing (n =30,
30 7, 2 and 1 samples, respectively), mainly due to lack of sample material or hemolysis.

31
32 Plasma samples were stored at -80°C until analysis. Glucose was analyzed immediately after sampling in
33 EDTA whole blood on HemoCue (HemoCue Denmark, Vedbaek, Denmark) and insulin was determined on
34 an Immulite 1000 analyzer (Siemens Medical Solutions Diagnostics, Los Angeles, Ca, USA) as described
35 elsewhere [29]. The limit of detection was 12 pmol/L in SKOT-I and 14 pmol/L in SKOT-II. Samples below the
36 detection limit for insulin were coded as 5.5 pmol/L (n=60) in SKOT-I and as 6.5 pmol/L (n=18) in SKOT-II.
37 Insulin resistance (IR) was estimated by the homeostasis model assessment (HOMA-IR) calculated as
38 $[\text{glucose (mmol/L)} \times \text{insulin (pmol/L)}]/135$ for SKOT-I and $[\text{glucose (mmol/L)} \times \text{insulin (pmol/L)}]/162$ for
39 SKOT-II due to new international standard. IGF-I and IGFBP-3 were assessed by automated

1 chemiluminescent immunoassay on Immulite 1000 (Diagnostic Products Corporation, Los Angeles, Ca, USA)
2 as described previously [28;38]. Detection limit for IGF-I was 25 ng/mL and samples below the detection
3 limit were coded as 12 ng/ml. There were n=20 and n=30 samples below the IGF-I detection limit for the
4 SKOT-I and SKOT-II cohorts, respectively. Adiponectin and leptin were analyzed using the human total
5 adiponectin and human leptin immunoassay Quantikine ELISA kit (R&D Systems Inc., Minneapolis, MN,
6 USA) with intra- and inter-assay CV of 4.7% and 6.7%, for leptin and 3.5% and 4.5% for adiponectin,
7 respectively.

8

9 **Background information**

10 Duration of exclusive and partial breastfeeding was recorded at the 9 months examination. Exclusive
11 breastfeeding was defined as receiving only breast milk, water and vitamins. Parental height and weight
12 were self-reported except for the SKOT-II mothers who were measured at the visit using a Tanita WB-
13 100MA (Tanita Corporation, Tokyo, Japan) and a 235 Heightronic Digital Stadiometer (QuickMedical,
14 Issaquah, WA, USA) for weight and height measurements, respectively. Information about pregnancy and
15 parental education was collected by questionnaires and interviews.

16

17 **Statistics**

18 Descriptive statistics are given by mean \pm SD or median and interquartile ranges (IQR) for normally or non-
19 normally distributed variables, respectively. Comparisons between genders were tested by independent *t*
20 test, Mann-Whitney U test or Chi-squared test as appropriate. Adjusted models for difference between
21 cohorts or between still breastfed and non-breastfed infants were performed using general linear models
22 (GLM). One model for difference between cohorts included a cohort \times current weight interaction term
23 which was removed if not significant. Outcome variables were glucose, insulin, HOMA-IR, IGF-I, IGFBP-3,
24 adiponectin and leptin. Bivariate correlations between outcomes were conducted using Spearman's rho.
25 Correlations between outcomes adjusted for gender were analyzed using log-transformed variables and
26 Pearson partial correlation. Associations between possible maternal, pregnancy and infant determinants
27 (mothers BMI at 9 months after birth, smoking in pregnancy, birth weight, infant weight and breastfeeding
28 status at 9 month) and each of the outcomes were investigated by GLM. The models were controlled for
29 gender, age at examination, GWG, education level of the mother, maternal age, duration of fasting and
30 energy in last meal. To explore correlations between outcomes and the influence of current breastfeeding,
31 and maternal, pregnancy and infant determinants on outcomes, the data from the two SKOT cohorts were
32 pooled and thus covering a larger range of the variables and an increased sample size. Residual plots and
33 Cooks distance were used for verification of GLM models and Levene's test for equal variance. Insulin,
34 HOMA-IR and leptin were log transformed and the estimates back transformed showing ratios. Significance
35 was defined as *p* values <0.05 and trends as *p* values <0.10. Data were analyzed using IBM SPSS Statistics
36 (Version 22, IBM, New York, NY).

1 **Results**

2 *Parental and infant body size by cohort*

3 Parental and infant characteristics are presented in Table 1. At birth, SKOT-II infants (obese mothers) were
4 4.4% (157g) heavier but not significantly longer than SKOT-I infants (population-based cohort). At age 9
5 months, SKOT-II infants were longer and heavier, before and after adjustment for age and gender, than
6 SKOT-I infants. There were however no differences in infant BMI or BMI-for-age z-scores, but surprisingly
7 SKOT-II infants had 1.0% (0.43 cm) smaller waist circumference. SKOT II infants were breastfed exclusively
8 for a shorter period than SKOT-I, and a lower percentage of SKOT II children (31.5%) were still breastfed at
9 9 months compared to the SKOT-I cohort (54.2%).

10 As expected maternal BMI at 9 months after birth was higher in SKOT-II compared to SKOT-I, and also
11 paternal BMI was considerably higher in SKOT-II. In SKOT-I, 19% of the mothers were overweight and 3%
12 obese, while these numbers were 9 % and 90% for mothers in SKOT-II. For the fathers in SKOT-I, 35% were
13 overweight and 9% obese, while for SKOT-II 45% of the fathers were overweight and 30% obese. Mothers
14 in SKOT-II had a 29% lower GWG and were 2 cm shorter than mothers in SKOT-I. Furthermore, the
15 education level was lower for both parents in SKOT-II.

16 *Infant biomarkers by cohort*

17

18 At 9 months, SKOT-II infants had higher values of insulin, HOMA-IR, leptin and adiponectin than SKOT-I
19 adjusted for gender and age (Table 2). Conversely, IGF-I levels were lower in SKOT-II than SKOT-I, and there
20 was no difference in IGFBP-3 or glucose. There was no difference in fasting time between the two cohorts
21 ($p=0.157$) but the energy content in the last meal before blood sampling was lower in SKOT-II (SKOT-II:
22 (median [IQR]) 492 [363-727] kJ; SKOT-I: 589 [385-839] kJ; $p=0.026$). However, the cohort differences in
23 insulin and HOMA-IR remained after control for energy content of last meal (data not shown).

24

25 In further models with additional adjustment for current weight, the cohort difference for leptin was
26 attenuated ($p=0.253$); but the other hormone differences persisted. Current weight was positively
27 associated with insulin ($p=0.027$), HOMA-IR ($p=0.022$), IGF-I ($p\leq 0.001$), IGFBP-3 ($p\leq 0.001$) and leptin
28 ($p\leq 0.001$). To explore if glucose, insulin resistance and hormone levels were modified differently by current
29 weight in the two cohorts, an interaction term between cohort and current weight was included in a final
30 model, but there was no significant interaction for any of the outcomes (all $p\geq 0.194$, data not shown).

31

32 *Correlations between biomarkers at 9 months*

33 In pooled data from SKOT-I and SKOT-II, leptin showed positive correlations with insulin, IGF-I and IGFBP-3,
34 and insulin was positively correlated with glucose, IGF-I and IGFBP-3 (Table 3). Adiponectin was not
35 correlated with any of the other hormones or glucose.

36 Girls had 24 %, 9.3% and 27% higher levels of IGF-I, IGFBP-3 and leptin respectively (all $p\leq 0.001$), but 3.4%
37 lower glucose concentration compared to boys ($p=0.002$). There was no difference for insulin, HOMA-IR
38 and adiponectin between genders (data not shown). Adjustment for gender did not substantially change
39 the inter-correlations between infant biomarkers (data not shown).

40

41

1 *Biomarkers and infant feeding*

2 Infants still breastfed compared to infant no longer breastfed at 9 months had lower levels of all the
3 hormones and HOMA-IR (adjusted for sex and age; Table 4) but there was no difference in glucose levels.
4 Additional adjustment for current weight attenuated the differences in leptin ($p=0.234$) and IGFBP-3
5 ($p=0.089$), but the lower levels of insulin, IGF-I, adiponectin and HOMA-IR persisted whereas glucose was
6 mildly higher in breastfed infants ($p=0.038$).

7

8

9 *Independent predictors of infant biomarkers at 9 months*

10 The independent impacts of maternal and pregnancy factors on infant glucose, insulin resistance and
11 hormone levels at 9 months were investigated using pooled data from SKOT-I and SKOT-II. Factors of
12 interest included in the multivariate regression analyses were birth weight, infant weight at 9 months,
13 infant feeding at 9 months, smoking during pregnancy and maternal BMI at 9 months after birth (Table 5).
14 The models were also adjusted for: gender, age at examination, GWG, maternal age, educational level of
15 the mother, duration of fasting and energy in last meal.

16

17 Maternal BMI was positively associated with insulin and adiponectin and negatively associated with IGF-I.
18 Birth weight was positively associated with leptin and negatively associated with IGF-I. Smoking during
19 pregnancy was positively associated with IGF-I at 9 months corresponding to 10.1 ng/mL lower IGF-I
20 concentration at 9 months in infants whose mothers did not smoke during pregnancy. Infants still breastfed
21 at 9 months had 1.36 pmol/L lower insulin, 1.32 lower HOMA-IR, 4.59 ng/mL lower IGF-I and 1.41 $\mu\text{g/mL}$
22 lower adiponectin levels than infants not breastfed. GWG was not significantly associated with any of the
23 hormones or glucose but tended to be positively associated with adiponectin ($p=0.068$).

24

25

26

1 Discussion

2 Maternal pre-pregnancy obesity seems to influence the metabolic profile in the offspring at 9 months of
3 age. We found that appetite- and growth related hormones were significantly different in infants born to
4 obese mothers (SKOT-II cohort) compared to infants born to mainly normal weight mothers (SKOT-I
5 cohort), showing elevated levels of insulin, adiponectin and leptin but surprisingly lower IGF-I
6 concentration. Furthermore, breastfeeding seemed to lower hormone levels at this age which is well into
7 the complementary feeding period.

8 The differences in hormone levels between the two cohorts were observed even though the BMI-z-scores
9 of the infants were similar. Adjustment for current weight weakened the significance only for leptin. This
10 was expected as leptin positively correlates with current body weight and BMI, and leptin is directly related
11 to body fat stores [33;39;40]. For insulin, the differences between cohorts could indicate some specific
12 change in infant insulin sensitivity independent of infant body size. The higher adiponectin levels in the
13 SKOT-II cohort could indicate a higher adipocyte number or size at this young age before adiponectin levels
14 become suppressed. Adiponectin is produced solely by adipocytes, in infancy circulating levels are relatively
15 high and are often positively correlated to body size [41], but in later childhood and adulthood circulating
16 levels decline and become inversely correlated with body size and leptin [21]. To our knowledge, our study
17 is the first to identify a possible direct influence of pre-pregnancy maternal BMI on offspring adiponectin
18 (independent of offspring body size).

19 The higher concentrations of insulin and leptin in our infants of obese mothers (SKOT-II) are consistent with
20 the over-nutrition hypothesis [6;8] and with findings of previous studies. Thus, pre-pregnancy obesity has
21 been associated with higher insulin and leptin concentration in cord blood at birth [42] and higher insulin
22 levels in later childhood as well as an adverse cardiometabolic profile and lower insulin sensitivity [3;43;44].
23 In contrast, Berglund et al. did not find any difference in cord blood insulin between infants born to obese
24 mothers and normal weight mothers but the sample size in that study was small [45]. The influence of
25 maternal overweight/obesity on leptin and adiponectin trajectories has been reported [17;34;46]. Volberg
26 et al. found no association between maternal pre-pregnancy BMI and offspring's leptin and adiponectin
27 trajectories up to 9 years [46]. They found that leptin were positively and adiponectin negatively
28 associated with the maternal pre-pregnancy BMI. However, adjustment for current weight seemed to
29 explain the associations [17]. Gruszfeld et al. found that maternal pre-pregnancy overweight was
30 associated with a high-increasing trajectory pattern up to 8 years for leptin but not for adiponectin [34].
31 Our study indicates that already in late infancy an impact of pre-pregnancy obesity can be observed in
32 offspring's hormone levels.

33 The literature on IGF-I concentrations during infancy in relation to pre-pregnancy maternal obesity is
34 limited. The lower IGF-I level in SKOT-II is highly robust ($P < 0.001$) but is opposite to what we had expected
35 for several reasons. SKOT-II infants were longer and heavier, and they were breastfed less than SKOT-I
36 infants. Furthermore, a previous cohort study from Turkey found a positive association between maternal
37 BMI and infant IGF-I cord blood levels [47], although other such studies found no association between cord
38 blood IGF-I and maternal obesity [48-50].

39 A previous study reported that pre-pregnancy maternal obesity was associated with higher infant weight-
40 for-length at 6 months [51] which indicates a positive influence of pre-pregnancy BMI on postnatal growth

1 but they did not report on IGF-I or insulin concentrations. In our study, there was no difference in infant
2 BMI between the cohorts and the mean BMI for age z-scores were in the normal range. This indicates that
3 the underlying cause for the difference in IGF-I levels in infancy between cohorts might be different than
4 the altered IGF-I levels observed in obese children compared to normal weight children [15;16].
5 Furthermore, the symmetrical larger body size (longer and heavier) of SKOT-II than SKOT-I infants at 9
6 months also supports some positive influence of maternal obesity on postnatal growth. The faster statural
7 growth in SKOT-II does not indicate greater height potential in these offspring, indeed their mothers were
8 shorter than SKOT-I mothers, but likely indicates a faster 'tempo' of infancy and childhood growth leading
9 to earlier pubertal maturation and no advantage for adult height [52]. In infancy, unlike during childhood,
10 statural growth is largely independent of growth hormone but rather is thought to be regulated by insulin-
11 dependent generation of IGF-I in response to nutrition [53]. The reason for the surprisingly lower IGF-I
12 levels in SKOT-II than SKOT-I infants is yet unclear; it could possibly reflect differences in IGF-I bioavailability
13 or some emerging defect in insulin signaling. We hypothesize that the apparent infant growth promoting
14 influence of maternal obesity may be driven by insulin acting independent of IGF-I.

15

16 Breastfeeding had marked apparent effects on lower growth- and appetite-related hormones analyzed in
17 this study. The effects were independent of current weight except for IGFBP-3 and leptin. Lower levels of
18 IGF-I and insulin in breastfed compared to formula fed infants are in accordance with other studies
19 investigating the influence of breastfeeding at different ages. Thus insulin and IGF-I levels were found to be
20 higher in formula fed infants in early infancy and for IGF-I also later in infancy [22;30;32;54]. A similar
21 pattern was reported for IGF-I in infants born to obese or overweight mothers [55].

22 Regarding leptin in breastfed versus formula fed infants conflicting results have been published. Consistent
23 with our findings, higher leptin levels in formula fed newborns (up to 5 days after delivery) and infants 3
24 months of age were reported [22;56]. No difference in leptin levels comparing breastfed to formula fed
25 infants has also been described [32;34] whereas Savino reported lower levels in formula fed than breastfed
26 children during infancy [18;30;57;58] but the sample size of these studies were small. However, we did not
27 find an independent effect as controlling for current weight explained the relation just as described above
28 for leptin. The level of adiponectin in breastfed versus formula fed infants is less studied. De Zegher et al.
29 measured the high-molecular-weight (HMW) adiponectin in infants born small for gestational age (SGA)
30 [59]. At 4 months of age the HMW adiponectin concentration was higher in formula fed than in breastfed
31 SGA infants, which is consistent with our findings (AGA) [59]. Together, these findings support the premise
32 that breastfeeding promotes an optimal (non-rapid) infant growth trajectory.

33 Smoking in pregnancy was associated with a higher IGF-I concentration at 9 months. This is in accordance
34 with the inverse relation of IGF-I and birth weight we also found, and could be explained by the mechanism
35 of subsequent catch up growth as seen in infants with low birth weight and infants of mothers smoking in
36 pregnancy [60;61]. The other hormones were not associated with smoking in pregnancy, but the power to
37 examine this was limited as only about 6% of the mothers were smoking during pregnancy.

38 Leptin was positive correlated with both insulin and IGF-I. This was expected as both are growth mediators
39 in infancy and leptin correlates with body weight. In literature conflicting results have been reported but
40 the studies also differ in age of the children, settings and methods [7;30;56;62]. Leptin was not related to

1 insulin at about 7 months of age [62] whereas leptin was positively associated with insulin at 1 year [7],
2 however both studies had a very small sample size. In newborns leptin and IGF-I were positively correlated
3 [56] whereas an inverse relation at 4 month was found in another study [30]. Adiponectin was not
4 correlated with any of the measured blood parameters which are in accordance with previous studies
5 [7;56;62].

6 The main strengths of this study are the relatively large number of infants with growth and metabolic
7 profiles obtained by combining two cohorts representing a wide range of maternal BMI. Furthermore, there
8 was a wide range in the parental education level representing different socioeconomic groups. Infants
9 born to obese mothers represent a group which often can be difficult to recruit to participate in scientific
10 studies whereas the SKOT-I families are characterized by high education and high income. Moreover the
11 study provides information on growth- and appetite related hormones measured simultaneously in healthy
12 infants and with detailed information on breastfeeding and maternal factors. The limitations of the study
13 includes that there were no measures of body fat mass, so though the BMI z-score of the cohorts were
14 comparable at 9 month, we do not know if the body composition differed. Furthermore, we had no data
15 on the pre-pregnancy BMI for the mothers in the SKOT-I cohort. However, the measured maternal BMI at 9
16 months after birth was very different for the two cohorts and presumably close to the pre-pregnancy
17 maternal BMI for SKOT-I as this seems to be the case for the SKOT II cohort, where 90% were still
18 categorized as obese and 9% as overweight. As it is an observational cohort study associations should be
19 interpreted with caution; there is a risk of residual confounding and no causative conclusions can be made.
20 In addition, this was an exploratory study so no correction for multiple testing was performed and
21 possibility of chance finding cannot be excluded.
22

23 In summary, infant offspring of obese mothers have an altered profile of growth and appetite related
24 hormones compared to offspring of non-obese mothers, with symmetrically larger infant body size and
25 higher levels of most growth and appetite-related hormones but surprisingly lower levels of IGF-I. The novel
26 link between maternal obesity on infant adiponectin levels and the possible infant growth promoting
27 effects of insulin, independent of IGF-I, should be investigated further.

28 **Acknowledgement**

29 The authors are grateful to participating children and caretakers. We would also like to thank project staff
30 for data collection and Vivian Anker and Inge Rasmussen for technical assistance. The SKOT-I study was
31 funded by The Directorate for Food, Fisheries and Agri Business as part of the project "Complementary and
32 young child feeding (CYCF) - impact on short and long term development and health". The SKOT-II study
33 was supported by grants from the Aase and Ejnar Danielsens Foundation and the Augustinus foundation
34 and partly by contributions from the research program 'Governing Obesity' by the University of
35 Copenhagen Excellence Program for Interdisciplinary (www.go.ku.dk). These studies are registered at
36 clinicaltrials.gov: SKOT I ([NCT02170428](https://clinicaltrials.gov/ct2/show/study/NCT02170428)) and SKOT II ([NCT02377973](https://clinicaltrials.gov/ct2/show/study/NCT02377973)). KKO is supported by the Medical
37 Research Council [Unit Programme MC_UU_12015/2].

38

39 Disclosure Statement

1 The authors have no conflicts of interest to disclose.

2 **Statement of Ethics**

3 Parents or custody holders of all participating infants provided written informed consent. The research was
4 ethically conducted in accordance with Declaration of Helsinki and approved by The Committees on
5 Biomedical Research Ethics for the Capital Region of Denmark.

6 7 **Author Contributions**

8 KFM and CM designed the study and supported data interpretation, EMC and KTE conducted the research,
9 AL designed the research, analyzed the data and prepared the first draft of the manuscript, KKO supported
10 decisions and interpretations regarding the analyses and initial draft preparation. All authors reviewed and
11 contributed to drafts and approved the final version of the manuscript.

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13 **References**

14

- 15 1 Bider-Canfield Z, Martinez MP, Wang X, Yu W, Bautista MP, Brookey J, Page KA, Buchanan TA,
16 Xiang AH: Maternal obesity, gestational diabetes, breastfeeding and childhood overweight at age
17 2 years. *Pediatr Obes* 2016.
- 18 2 Whitaker RC: Predicting preschooler obesity at birth: the role of maternal obesity in early
19 pregnancy. *Pediatrics* 2004;114:e29-e36.
- 20 3 Gaillard R, Steegers EA, Duijts L, Felix JF, Hofman A, Franco OH, Jaddoe VW: Childhood
21 cardiometabolic outcomes of maternal obesity during pregnancy: the Generation R Study.
22 *Hypertension* 2014;63:683-691.
- 23 4 Yu Z, Han S, Zhu J, Sun X, Ji C, Guo X: Pre-pregnancy body mass index in relation to infant birth
24 weight and offspring overweight/obesity: a systematic review and meta-analysis. *PLoS One*
25 2013;8:e61627.
- 26 5 Cooper R, Hypponen E, Berry D, Power C: Associations between parental and offspring adiposity
27 up to midlife: the contribution of adult lifestyle factors in the 1958 British Birth Cohort Study. *Am*
28 *J Clin Nutr* 2010;92:946-953.
- 29 6 Lawlor DA, Smith GD, O'Callaghan M, Alati R, Mamun AA, Williams GM, Najman JM:
30 Epidemiologic evidence for the fetal overnutrition hypothesis: findings from the mater-university
31 study of pregnancy and its outcomes. *Am J Epidemiol* 2007;165:418-424.
- 32 7 Iniguez G, Soto N, Avila A, Salazar T, Ong K, Dunger D, Mericq V: Adiponectin levels in the first
33 two years of life in a prospective cohort: relations with weight gain, leptin levels and insulin
34 sensitivity. *J Clin Endocrinol Metab* 2004;89:5500-5503.
- 35 8 Dabelea D, Crume T: Maternal environment and the transgenerational cycle of obesity and
36 diabetes. *Diabetes* 2011;60:1849-1855.

- 1 9 Stettler N, Zemel BS, Kumanyika S, Stallings VA: Infant weight gain and childhood overweight
2 status in a multicenter, cohort study. *Pediatrics* 2002;109:194-199.
- 3 10 Sachdev HS, Fall CH, Osmond C, Lakshmy R, Dey Biswas SK, Leary SD, Reddy KS, Barker DJ,
4 Bhargava SK: Anthropometric indicators of body composition in young adults: relation to size at
5 birth and serial measurements of body mass index in childhood in the New Delhi birth cohort. *Am*
6 *J Clin Nutr* 2005;82:456-466.
- 7 11 Demerath EW, Reed D, Choh AC, Soloway L, Lee M, Czerwinski SA, Chumlea WC, Siervogel RM,
8 Towne B: Rapid Postnatal Weight Gain and Visceral Adiposity in Adulthood: The Fels Longitudinal
9 Study. *Obesity (Silver Spring)* 2009;17:2060-2066.
- 10 12 Leunissen RW, Kerkhof GF, Stijnen T, Hokken-Koelega A: Timing and tempo of first-year rapid
11 growth in relation to cardiovascular and metabolic risk profile in early adulthood. *JAMA*
12 2009;301:2234-2242.
- 13 13 Savastano S, Di SC, Barrea L, Colao A: The complex relationship between obesity and the
14 somatotropic axis: the long and winding road. *Growth Horm IGF Res* 2014;24:221-226.
- 15 14 Lewitt MS, Dent MS, Hall K: The Insulin-Like Growth Factor System in Obesity, Insulin Resistance
16 and Type 2 Diabetes Mellitus. *J Clin Med* 2014;3:1561-1574.
- 17 15 Street ME, Smerieri A, Montanini L, Predieri B, Iughetti L, Valenzise M, De LF, Vigone M, Weber G,
18 Maghnie M, Bernasconi S: Interactions among pro-inflammatory cytokines, IGF system and
19 thyroid function in pre-pubertal obese subjects. *J Biol Regul Homeost Agents* 2013;27:259-266.
- 20 16 Ricco RC, Ricco RG, Queluz MC, de Paula MTS, Atique PV, Custodio RJ, Tourinho FH, Del Roio LR,
21 Jr., Martinelli CE, Jr.: IGF-1R mRNA expression is increased in obese children. *Growth Horm IGF*
22 *Res* 2018;39:1-5.
- 23 17 Volberg V, Harley KG, Aguilar RS, Rosas LG, Huen K, Yousefi P, Dave V, Phan N, Lustig RH, Eskenazi
24 B, Holland N: Associations between perinatal factors and adiponectin and leptin in 9-year-old
25 Mexican-American children. *Pediatr Obes* 2013;8:454-463.
- 26 18 Savino F, Liguori SA, Oggero R, Silvestro L, Miniero R: Maternal BMI and serum leptin
27 concentration of infants in the first year of life. *Acta Paediatr* 2006;95:414-418.
- 28 19 Mantzoros CS, Magkos F, Brinkoetter M, Sienkiewicz E, Dardeno TA, Kim SY, Hamnvik OP, Koniaris
29 A: Leptin in human physiology and pathophysiology. *Am J Physiol Endocrinol Metab*
30 2011;301:E567-E584.
- 31 20 Bever BJ, Reifsnider E, Mendias E, Moramarco MW, Davila YR: Reduced breastfeeding rates
32 among obese mothers: a review of contributing factors, clinical considerations and future
33 directions. *Int Breastfeed J* 2015;10:21.
- 34 21 Savino F, Petrucci E, Nanni G: Adiponectin: an intriguing hormone for paediatricians. *Acta*
35 *Paediatr* 2008;97:701-705.
- 36 22 Breij LM, Mulder MT, van Vark-van der Zee LC, Hokken-Koelega AC: Appetite-regulating
37 hormones in early life and relationships with type of feeding and body composition in healthy
38 term infants. *Eur J Nutr* 2016;56:1725-1732.

- 1 23 Horta BL, Loret de MC, Victora CG: Long-term consequences of breastfeeding on cholesterol,
2 obesity, systolic blood pressure and type 2 diabetes: a systematic review and meta-analysis. *Acta*
3 *Paediatr* 2015;104:30-37.
- 4 24 Yan J, Liu L, Zhu Y, Huang G, Wang PP: The association between breastfeeding and childhood
5 obesity: a meta-analysis. *BMC Public Health* 2014;14:1267.
- 6 25 Dewey KG, Heinig MJ, Nommsen LA, Peerson JM, Lonnerdal B: Growth of breast-fed and formula-
7 fed infants from 0 to 18 months: the DARLING Study. *Pediatrics* 1992;89:1035-1041.
- 8 26 Roche AF, Guo S, Siervogel RM, Khamis HJ, Chandra RK: Growth comparison of breast-fed and
9 formula-fed infants. *Can J Public Health* 1993;84:132-135.
- 10 27 Koletzko B, von KR, Cloasa R, Escribano J, Scaglioni S, Giovannini M, Beyer J, Demmelmair H,
11 Gruszfeld D, Dobrzanska A, Sengier A, Langhendries JP, Rolland Cachera MF, Grote V: Lower
12 protein in infant formula is associated with lower weight up to age 2 y: a randomized clinical trial.
13 *Am J Clin Nutr* 2009;89:1836-1845.
- 14 28 Madsen AL, Larnkjær A, Mølgaard C, Michaelsen KF: IGF-I and IGFBP-3 in healthy 9month old
15 infants from the SKOT cohort: Breastfeeding, diet, and later obesity. *Growth Horm IGF Res*
16 2011;21:199-204.
- 17 29 Madsen AL, Schack-Nielsen L, Larnkjaer A, Mølgaard C, Michaelsen KF: Determinants of blood
18 glucose and insulin in healthy 9-month-old term Danish infants; the SKOT cohort. *Diabet Med*
19 2010;27:1350-1357.
- 20 30 Savino F, Fissore MF, Grassino EC, Nanni GE, Oggero R, Silvestro L: Ghrelin, leptin and IGF-I levels
21 in breast-fed and formula-fed infants in the first years of life. *Acta Paediatr* 2005;94:531-537.
- 22 31 Lonnerdal B, Havel PJ: Serum leptin concentrations in infants: effects of diet, sex, and adiposity.
23 *Am J Clin Nutr* 2000;72:484-489.
- 24 32 Inostroza J, Haschke F, Steenhout P, Grathwohl D, Nelson SE, Ziegler EE: Low-protein formula
25 slows weight gain in infants of overweight mothers. *J Pediatr Gastroenterol Nutr* 2014;59:70-77.
- 26 33 Savino F, Sardo A, Rossi L, Benetti S, Savino A, Silvestro L: Mother and Infant Body Mass Index,
27 Breast Milk Leptin and Their Serum Leptin Values. *Nutrients* 2016;8.
- 28 34 Gruszfeld D, Kulaga Z, Wierzbicka A, Rzehak P, Grote V, Martin F, Poncelet P, Cloasa-Monasterolo
29 R, Escribano J, Verduci E, Riva E, Koletzko B: Leptin and Adiponectin Serum Levels from Infancy to
30 School Age: Factors Influencing Tracking. *Child Obes* 2016;12:179-187.
- 31 35 Andersen LB, Pippert CB, Trolle E, Bro R, Larnkjaer A, Carlsen EM, Molgaard C, Michaelsen KF:
32 Maternal obesity and offspring dietary patterns at 9 months of age. *Eur J Clin Nutr* 2015;69:668-
33 675.
- 34 36 Renault KM, Norgaard K, Nilas L, Carlsen EM, Cortes D, Pryds O, Secher NJ: The Treatment of
35 Obese Pregnant Women (TOP) study: a randomized controlled trial of the effect of physical
36 activity intervention assessed by pedometer with or without dietary intervention in obese
37 pregnant women. *Am J Obstet Gynecol* 2014;210:134-139.

- 1 37 WHO: World Health Organization. The WHO Child Growth Standards; WHO Anthro 2005; 2007.
- 2 38 Ejlerskov KT, Larnkjaer A, Pedersen D, Ritz C, Molgaard C, Michaelsen KF: IGF-I at 9 and 36
3 months of age - relations with body composition and diet at 3 years - the SKOT cohort. *Growth*
4 *Horm IGF Res* 2014;24:239-244.
- 5 39 Wilasco MI, Goldani HA, Dornelles CT, Maurer RL, Kieling CO, Porowski M, Silveira TR: Ghrelin,
6 leptin and insulin in healthy children: Relationship with anthropometry, gender, and age
7 distribution. *Regul Pept* 2012;173:21-26.
- 8 40 Nagy TR, Gower BA, Trowbridge CA, Dezenberg C, Shewchuk RM, Goran MI: Effects of gender,
9 ethnicity, body composition, and fat distribution on serum leptin concentrations in children. *J Clin*
10 *Endocrinol Metab* 1997;82:2148-2152.
- 11 41 Dunger D, Ong K: Abundance of adiponectin in the newborn. *Clin Endocrinol (Oxf)* 2004;61:416-
12 417.
- 13 42 Kaar JL, Brinton JT, Crume T, Hamman RF, Glueck DH, Dabelea D: Leptin levels at birth and infant
14 growth: the EPOCH study. *J Dev Orig Health Dis* 2014;5:214-218.
- 15 43 Tan HC, Roberts J, Catov J, Krishnamurthy R, Shypailo R, Bacha F: Mother's pre-pregnancy BMI is
16 an important determinant of adverse cardiometabolic risk in childhood. *Pediatr Diabetes*
17 2015;16:419-426.
- 18 44 Derraik JG, Ayyavoo A, Hofman PL, Biggs JB, Cutfield WS: Increasing maternal prepregnancy body
19 mass index is associated with reduced insulin sensitivity and increased blood pressure in their
20 children. *Clin Endocrinol (Oxf)* 2015;83:352-356.
- 21 45 Berglund SK, Garcia-Valdes L, Torres-Espinola FJ, Segura MT, Martinez-Zaldivar C, Aguilar MJ, Agil
22 A, Lorente JA, Florido J, Padilla C, Altmae S, Marcos A, Lopez-Sabater MC, Campoy C: Maternal,
23 fetal and perinatal alterations associated with obesity, overweight and gestational diabetes: an
24 observational cohort study (PREOBE). *BMC Public Health* 2016;16:207.
- 25 46 Volberg V, Heggseth B, Harley K, Huen K, Yousefi P, Dave V, Tyler K, Vedar M, Eskenazi B,
26 Holland N: Adiponectin and leptin trajectories in Mexican-American children from birth to 9 years
27 of age. *PLoS One* 2013;8:e77964.
- 28 47 Akcakus M, Koklu E, Kurtoglu S, Kula M, Koklu SS: The relationship among intrauterine growth,
29 insulinlike growth factor I (IGF-I), IGF-binding protein-3, and bone mineral status in newborn
30 infants. *Am J Perinatol* 2006;23:473-480.
- 31 48 Regnault N, Botton J, Heude B, Forhan A, Hankard R, Foliguet B, Hillier TA, Souberbielle JC,
32 Dargent-Molina P, Charles MA: Higher cord C-peptide concentrations are associated with slower
33 growth rate in the 1st year of life in girls but not in boys. *Diabetes* 2011;60:2152-2159.
- 34 49 Ferraro ZM, Qiu Q, Gruslin A, Adamo KB: Characterization of the insulin-like growth factor axis in
35 term pregnancies complicated by maternal obesity. *Hum Reprod* 2012;27:2467-2475.
- 36 50 Skalkidou A, Petridou E, Papathoma E, Salvanos H, Kedikoglou S, Chrousos G, Trichopoulos D:
37 Determinants and consequences of major insulin-like growth factor components among full-term
38 healthy neonates. *Cancer Epidemiol Biomarkers Prev* 2003;12:860-865.

- 1 51 Deierlein AL, Siega-Riz AM, Adair LS, Herring AH: Effects of pre-pregnancy body mass index and
2 gestational weight gain on infant anthropometric outcomes. *J Pediatr* 2011;158:221-226.
- 3 52 Ahmed ML, Ong KK, Dunger DB: Childhood obesity and the timing of puberty. *Trends Endocrinol*
4 *Metab* 2009;20:237-242.
- 5 53 Hill DJ, Hogg J: Growth factors and the regulation of pre- and postnatal growth. *Baillieres Clin*
6 *Endocrinol Metab* 1989;3:579-625.
- 7 54 Putet G, Labaune JM, Mace K, Steenhout P, Grathwohl D, Raverot V, Morel Y, Picaud JC: Effect of
8 dietary protein on plasma insulin-like growth factor-1, growth, and body composition in healthy
9 term infants: a randomised, double-blind, controlled trial (Early Protein and Obesity in Childhood
10 (EPOCH) study). *Br J Nutr* 2016;115:271-284.
- 11 55 Martin FP, Moco S, Montoliu I, Collino S, Da SL, Rezzi S, Prieto R, Kussmann M, Inostroza J,
12 Steenhout P: Impact of breast-feeding and high- and low-protein formula on the metabolism and
13 growth of infants from overweight and obese mothers. *Pediatr Res* 2014;75:535-543.
- 14 56 Petridou E, Mantzoros CS, Belechri M, Skalkidou A, Dessypris N, Papatoma E, Salvanos H, Lee JH,
15 Kedikoglou S, Chrousos G, Trichopoulos D: Neonatal leptin levels are strongly associated with
16 female gender, birth length, IGF-I levels and formula feeding. *Clin Endocrinol (Oxf)* 2005;62:366-
17 371.
- 18 57 Savino F, Costamagna M, Prino A, Oggero R, Silvestro L: Leptin levels in breast-fed and formula-
19 fed infants. *Acta Paediatr* 2002;91:897-902.
- 20 58 Savino F, Liguori SA, Benetti S, Sorrenti M, Fissore MF, Cordero di ML: High serum leptin levels in
21 infancy can potentially predict obesity in childhood, especially in formula-fed infants. *Acta*
22 *Paediatr* 2013;102:e455-e459.
- 23 59 de ZF, Sebastiani G, Diaz M, Sanchez-Infantes D, Lopez-Bermejo A, Ibanez L: Body composition
24 and circulating high-molecular-weight adiponectin and IGF-I in infants born small for gestational
25 age: breast- versus formula-feeding. *Diabetes* 2012;61:1969-1973.
- 26 60 Nohr EA, Vaeth M, Baker JL, Sorensen TI, Olsen J, Rasmussen KM: Pregnancy outcomes related to
27 gestational weight gain in women defined by their body mass index, parity, height, and smoking
28 status. *Am J Clin Nutr* 2009;90:1288-1294.
- 29 61 Martin A, Connelly A, Bland RM, Reilly JJ: Health impact of catch-up growth in low-birth weight
30 infants: systematic review, evidence appraisal, and meta-analysis. *Matern Child Nutr* 2017;13.
- 31 62 Diamon F, Dharamraj C, Luther S, Eichler D: The leptin/adiponectin ratio in mid-infancy correlates
32 with weight gain in healthy term infants, but is unrelated to serum insulin concentrations, body
33 mass index, or skin fold thickness. *J Pediatr Endocrinol Metab* 2008;21:1133-1138.
34
35