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

Anni Larnkjær[1], Ken K Ong[2,3], Emma M Carlsen[1,4], Katrine T Ejlerskov[1], Christian Mølgaard[1] and Kim F. Michaelsen[1]

Affiliations: [1] Department of Nutrition, Exercise and Sports, Faculty of Science, University of Copenhagen, Denmark, [2,] MRC Epidemiology Unit, Institute of Metabolic Science, University of Cambridge, Cambridge, UK, [3] Department of Paediatrics, University of Cambridge, Cambridge, UK, [4]Department of Pediatrics, Copenhagen University Hospital Hvidovre, Hvidovre, Denmark

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Corresponding author:

Anni Larnkjær

Rolighedsvej 26,

DK-1958 Frederiksberg C,

Denmark

Phone +45 35333548, (2493 secr.), fax +45 35332469,

E-mail: ala@nexs.ku.dk

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Abstract

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

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

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

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

Introduction

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

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

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

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

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

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

Subjects and Methods

Study design and participants

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

Anthropometric measurements

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

Blood samples

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

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

Plasma samples were stored at -80°C until analysis. Glucose was analyzed immediately after sampling in EDTA whole blood on HemoCue (HemoCue Denmark, Vedbaek, Denmark) and insulin was determined on an Immulite 1000 analyzer (Siemens Medical Solutions Diagnostics, Los Angeles, Ca, USA) as described elsewhere [29]. The limit of detection was 12 pmol/L in SKOT-I and 14 pmol/L in SKOT-II. Samples below the 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. Insulin resistance (IR) was estimated by the homeostasis model assessment (HOMA-IR) calculated as [glucose (mmol/L) × insulin (pmol/L)]/135 for SKOT-I and [glucose (mmol/L) × insulin (pmol/L)]/162 for SKOT-II due to new international standard. IGF-I and IGFBP-3 were assessed by automated

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

Background information

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

Statistics

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

Results

Parental and infant body size by cohort

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

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

Infant biomarkers by cohort

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

In further models with additional adjustment for current weight, the cohort difference for leptin was attenuated ($p=0.253$); but the other hormone differences persisted. Current weight was positively associated with insulin ($p=0.027$), HOMA-IR ($p=0.022$), IGF-I ($p\leq0.001$), IGFBP-3 ($p\leq0.001$) and leptin ($p\leq0.001$). To explore if glucose, insulin resistance and hormone levels were modified differently by current weight in the two cohorts, an interaction term between cohort and current weight was included in a final model, but there was no significant interaction for any of the outcomes (all $p\geq 0.194$, data not shown).

Correlations between biomarkers at 9 months

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

Girls had 24 %, 9.3% and 27% higher levels of IGF-I, IGFBP-3 and leptin respectively (all $p\leq0.001$), but 3.4% lower glucose concentration compared to boys ($p=0.002$). There was no difference for insulin, HOMA-IR and adiponectin between genders (data not shown). Adjustment for gender did not substantially change the inter-correlations between infant biomarkers (data not shown).

Biomarkers and infant feeding

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

Independent predictors of infant biomarkers at 9 months

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

Maternal BMI was positively associated with insulin and adiponectin and negatively associated with IGF-I. Birth weight was positively associated with leptin and negatively associated with IGF-I. Smoking during pregnancy was positively associated with IGF-I at 9 months corresponding to 10.1 ng/mL lower IGF-I concentration at 9 months in infants whose mothers did not smoke during pregnancy. Infants still breastfed 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 μg/mL lower adiponectin levels than infants not breastfed. GWG was not significantly associated with any of the hormones or glucose but tended to be positively associated with adiponectin (p=0.068).

Discussion

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

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

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

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

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

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

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

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

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

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

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

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

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

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Disclosure Statement

The authors have no conflicts of interest to disclose.

Statement of Ethics

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

Author Contributions

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

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