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Adipokines in umbilical cord blood from children born large for gestational age

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Abstract

Background: The etiology of childhood obesity and the associated morbidity is multifactorial. Recently, data suggesting a prenatal programming towards later childhood obesity and metabolic deregulation through the intrauterine environment has emerged. This study explored the concentrations of adipokines and their mutual relationship at birth in children born to non-diabetic mothers.
Methods: Adiponectin, leptin and sOB-R were measured using ELISA-based commercial kits in umbilical cord blood from 60 neonates (30 born large for gestational age [LGA] and 30 born appropriate for gestational age [AGA]). Children exposed to maternal diabetes, chronic disease and preeclampsia were excluded.
Results: The LGA group exhibited significantly elevated concentrations of leptin ($p<0.001$) and of free leptin index ($p<0.001$) and decreased sOB-R concentrations ($p=0.005$) when compared to the AGA group, which persisted in multiple regression analysis after taking the gestational age into account ($p=0.048$, $p<0.001$ and $p<0.001$, respectively). Only a trend towards a difference in adiponectin was demonstrated ($p=0.057$) regardless of adjustment ($p=0.150$). However, the leptin/adiponectin ratio was elevated in the LGA group ($p=0.008$), regardless of adjustment ($p=0.039$).
Conclusion: The data indicate a disturbance of adipokines in macrosomic newborns and that the mutual ratios between adipokines may provide a more sensitive marker of metabolic disturbance than any isolated adipokine.

Keywords: adipokines; adiponectin; birth weight; free-leptin index; large for gestational age; leptin; soluble leptin receptor.

Introduction

In recent years childhood obesity has increased to pan-epidemic proportions along with a concomitant increase in obesity-associated morbidity (1). The etiology of childhood obesity is complex and multifactorial and has among others been linked to genetic, metabolic, nutritional, activity, socioeconomic, psychological and prenatal factors (1).

The prenatal life is a time of rapid cellular growth and replication and of functional maturation of organs, and these processes are sensitive to disturbances in the intrauterine milieu. Accordingly, there is increasing evidence supporting the developmental origins of a health and disease hypothesis that suggests a prenatal programming towards later childhood obesity and metabolic dysregulation through the intrauterine environment (2, 3). Consequently, infants that are born large for gestational age (LGA) are more likely to be obese in childhood and adolescence (4–6) and are at risk of cardiovascular and metabolic obesity-related complications later in life (7).

LGA is often defined as a birth weight above the 90th percentile for gestational age and gender (8), and although some of these neonates are simply constitutionally large, LGA babies have significantly higher morbidity than appropriate-for-gestational-age (AGA) babies (8). There are continuous associations between maternal glucose levels and a variety of adverse outcomes, including LGA, and these outcomes are not confined to diabetic pregnancies (9). It is plausible that fetal programming of

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metabolic dysfunction is a continuous relationship not solely occurring in diabetic pregnancies and accordingly that a far greater proportion of pregnancies may be at risk (10).

Adiponectin is an adipocyte-secreted hormone that modulates a number of metabolic processes, including glucose regulation and fatty-acid oxidation, and has an insulin-sensitizing effect (11, 12). It is the most abundant adipokine, and interestingly, studies in adults have demonstrated an inverse correlation between hormone secretion and glandular hypertrophy as serum adiponectin levels decrease with increasing fat tissue (13), whereas the correlation between birth weight and umbilical cord adiponectin levels remains debatable (14–16). The adiponectin level in umbilical cord blood has been demonstrated to be influenced by maternal diabetic state with lower levels in neonates born to diabetic mothers (16). Recently, adiponectin has gained increasing attention in pediatrics as a serum biomarker due to its correlation to various metabolic risk factors including cardiovascular disease (17, 18), and interestingly, adiponectin levels in children have been demonstrated to be associated to preceding birth weight (19).

Leptin is another adipokine involved in the regulation of feeding and energy balance and serves as a mediator to the neuroendocrinological adaptation to fasting (20). Leptin appears to play a role in a number of processes associated with pathological fetal growth, most notably maternal diabetes (21, 22). Leptin in umbilical cord blood has been demonstrated to be of fetal origin as it is independent of maternal levels of leptin (23). Leptin has recently been proposed as a biomarker of fetal adiposity (24) and may provide a biological link for the fetal programming of later adult metabolic and cardiovascular health (10). The bioavailability of leptin is modulated by the soluble leptin receptor (sOB-R) (25), and the sOB-R has also been inversely linked to birth weight (26) and to intrauterine growth restriction (27). It has been proposed that the balance between leptin and sOB-R, or the free leptin index (FLI), might be a useful tool to assess leptin activity (28). Similarly, the leptin/adiponectin ratio has demonstrated stronger correlation to body fat percent than adiponectin alone in children (29).

The role of adipokines in LGA babies born to nondiabetic mothers is not fully explored. The present study aims to elucidate the relationship between birth weight and adipokines by investigating the adiponectin, leptin and sOB-R concentrations and their mutual relationship, expressed as FLI and adiponectin/leptin ratio, in the cord blood from children born LGA compared to AGA newborns.

Materials and methods

Subjects

Umbilical cord blood samples were collected from term or near-term (gestational age, GA, >36 weeks) children born LGA and AGA at the Clinic of Obstetrics, Copenhagen University Hospital, Denmark. The initial sampling was originally collected and performed as part of studies investigating preleukemic translocations in newborns (30, 31). Maternal, obstetric and perinatal data were extracted from hospital files. Gestational age was estimated by first trimester ultrasound evaluations that allow for estimation of accurate term within 4.7 days with a 95% probability (32).

The infants were defined LGA or AGA by comparing their actual birth weight to the expected birth weight that was calculated by using previously published curves for ultrasound-based fetal weight references from a Scandinavian population (33). LGA was defined as above the 90th percentile expected for term and gender (8). In order to obtain a relatively homogenous group of infants, children who were exposed to maternal chronic disease, diabetes and preeclampsia were excluded. Finally, we excluded all children with neonatal depression, i.e. all included children had an Apgar score >7 at 5 min.

To obtain as ideal a control group as possible, an equal number of AGA children with birth weight close to the expected birth weight were randomly selected and included.

Adipokines

Umbilical cord blood was drawn from the placenta immediately after delivery and collected in 3-mL ethylene diamine tetra-acetic acid tubes and stored at room temperature until processing. As cytokines are degraded over time, plasma was collected within 12 h, which is within the known stability time of adiponectin (34) and leptin (35), and subsequently stored at −80°C until further handling. Total adiponectin, leptin and the sOB-R were measured using DuoSet ELISA kit (R&D Systems, Garland, TX, USA) according to the manufacturer's protocol and run on a Victor 3 Multilabel counter 1420 platform (Perkin Elmer, Waltham, MA, USA). As levels of sOB-R can provide an indication of free leptin, the FLI being defined as the ratio of leptin to sOB-R (36) was calculated.

Analyses

The R statistical software version 3.1.0 (R Foundation for Statistical Computing, Vienna, Austria) was used for statistical analyses. Comparison of groups was done by Mann-Whitney U-test. The associations between birth weight and the various adipokines were analyzed in multiple linear regression models adjusted for gestational age. A p value of <0.05 was considered significant.

Ethics

All participating mothers signed informed consent. For ethical reasons, all samples were blinded, and renewed contact to the families

was not allowed. The study was approved by the Danish Data Protection Agency and Scientific Ethics Committee.

Results

A total of 60 infants comprising 30 SGA and 30 AGA infants were included in the present study. The baseline characteristics of both groups are presented in Table 1. The patients did not differ in terms of maternal age, gravidity, gender, gestational age or Apgar score. The LGA newborns were significantly larger in terms of birth weight and length, cranial and abdominal circumference, ponderal index and birth weight SDS. All cytokines were within optimum detection range (data not shown).

Significant elevations in leptin was demonstrated in the LGA group ($p<0.001$) compared to the AGA group, which persisted in a multiple regression analysis after taking the gestational age into account ($p=0.048$). A significant decrease in sOB-R was found in the LGA group ($p=0.005$), and in multiple regression analysis taking into account the gestational age, this difference persisted ($p<0.001$). Only a tendency towards a difference in adiponectin between the two groups was demonstrated ($p=0.057$) regardless of adjustment ($p=0.150$).

The LGA children were found to have a significantly elevated FLI ($p<0.001$), which persisted when adjusted for gestational age ($p<0.001$). Similarly, the LGA were found to have a significantly decreased adiponectin/leptin ratio ($p=0.008$), regardless of adjustment ($p=0.039$).

Discussion

The leptin system is now well established as contributing to fetal growth, and several studies have reported a positive association of leptin concentrations on birth indices (37–39), but most studies have not evaluated associations with the sOB-R (40). In the presented study, leptin was found in significantly higher levels in LGA neonates when compared to the AGA group, a finding that complies with previous findings (41, 42). The sOB-R modulates the bioavailability of leptin, and we found that the concentrations of the sOB-R were lower in the LGA group. Relatively few studies on the subject have taken the sOB-R into account, but our findings support the previously demonstrated negative association between sOB-R and birth weight (40). Furthermore, we demonstrated significant elevation in the free leptin ratio, which may suggest that free leptin is a better marker of fetal adiposity than leptin alone.

Adiponectin is well described as associated to birth weight as several studies have demonstrated a positive correlation between cord blood adiponectin and birth weight (41, 43) while others have found a negative association, when examining macrosomic newborns (16, 42).

Table 1: Baseline characteristics and correlation of adipokines between the groups.

Baseline characteristics	LGA (n=30)	AGA (n=30)	p-Value[a]
Maternal age, years	30.04 (24.64; 37.00)	30.97 (24.60; 43.11)	0.491
Gravidity	2 (1; 4)	1 (1; 5)	0.389
Parity	2 (1; 4)	1 (1; 4)	0.015
Gender, M:F	17:13	14:16	0.605[b]
Gestational age, week+days	39+1 (36+2; 42+3)	39+3 (36+5; 42+1)	0.118
Birth weight, g	4472 (3596; 5429)	3459 (2890; 3936)	<0.001
Birth length, cm	54 (49; 60)	51 (48; 54)	<0.001
Head circumference, cm	36 (34; 39)	34 (31; 39)	<0.001
Abdominal circumference, cm	35 (32; 40)	34 (31; 39)	<0.001
BWfGA, SDS	1.377 (1.283; 2.465)	0.001 (–0.229; 0.255)	<0.001
PI, kg/m^3	28.47 (23.80; 38.61)	25.82 (21.62; 32.38)	0.001
5-min Apgar	10 (10; 10)	10 (10; 10)	n/a
Adipocytokine			
sOB-R, ng/mL	16.10 (6.33; 30.86)	21.39 (9.77; 51.08)	0.005
Leptin, ng/mL	23.15 (6.19; 88.65)	12.07 (0.39; 70.62)	<0.001
Adiponectin, µg/mL	13.88 (4.46;35.7)	11.33 (2.05; 25.52)	0.057
Free leptin ratio	1363.4 (282.8; 7350.7)	512.7 (22.4; 3207.1)	<0.001
Adiponectin/leptin ratio	1.542 (0.583; 5.302)	1.075 (0.075; 3.967)	0.008

AGA, appropriate for gestational age; BWfGA, birth weight for gestational age; F, female; LGA, large for gestational age; M, male; PI, ponderal index; SDS, standard deviations score; sOB-R, soluble leptin receptor. Numbers are medians and range. [a]Mann-Witney (see text for details). [b]χ^2.

Yet, only a borderline difference in the adiponectin levels between the LGA and the AGA groups with the former exhibiting the highest concentrations was found in the present study. This may reflect that only term or near-term newborns were included. Additionally, the association was further weakened when taking the gestational age into account, as is also seen in other studies (42, 44). However, in the present study, the leptin/adiponectin ratio was significantly elevated in the LGA group, and it is possible that this ratio may be a better marker of overnutritional status in newborns than adiponectin alone.

The macrosomic fetus is defined as one that has exceeded its genetically determined growth potential regardless of etiological cause, whereas a LGA fetus is defined as above a specified birth weight percentile (45). The present study draws strength from the use of ultrasound-based fetal weight references from a Scandinavian population (33) in an attempt to take biological variation in birth weight depending on ethnicity and gender into account. For ethical reasons no registration on ethnicity was made in this study, but the included cases are likely to be more coherent with the Scandinavian fetal weight references than the WHO references. However, regardless of cutoff, it is generally believed that that some LGA children are structurally large and therefore do not suffer from disturbed growth (8). It is therefore plausible that a 'true' cohort of growth-disturbed newborn would exhibit even larger disturbances in adipokine levels.

It has been demonstrated that cord blood concentrations of leptin and adiponectin are associated to subsequent BMI and central adiposity in children (21). Therefore, whereas the clinical applications of this data remain speculative, it may be possible that neonatal levels of adipokines and particularly their mutual relationship could be used to identify neonates with increased risk of developing childhood obesity and/or its related complications.

Altogether, the results of the present study add to the increasing data indicating a disturbance of adipokines in newborns exhibiting disturbed growth even in children born to non-diabetic mothers. However, whether the observed disturbance in adipokines is an epiphenomenon to a macrosomic state or simply a biological variation in the intra-uterine growth balance remains to be elucidated.

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Conflict of interest statement: The authors declare no conflicts of interest.

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