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Cord blood nesfatin-1 in large for gestational age pregnancies

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ABSTRACT

Objective: To investigate possible alterations in cord blood levels of adipokine nesfatin-1 (secreted by adipose tissue and pancreatic β-cells and implicated in glucose metabolism and insulin resistance), as well as insulin, in large (LGA) and appropriate for gestational age (AGA) pregnancies, granted that these groups differ in body fat mass and metabolic/endocrine mechanisms.

Materials and methods: Cord blood nesfatin-1 and insulin concentrations were prospectively measured in 40 LGA (9 born from diabetic and 31 from non-diabetic mothers) and 20 AGA singleton full-term infants as well as their mothers.

Results: Cord blood nesfatin-1 concentrations were significantly lower in LGA compared to AGA neonates ($b = -0.206$, SE 0.07, $p = 0.005$). However, cord blood nesfatin-1 concentrations were elevated in infants born from mothers with gestational diabetes mellitus (GDM), compared to those born from non-diabetic mothers, after controlling for group ($b = 0.190$, SE 0.10, $p = 0.05$). Finally, cord blood nesfatin-1 concentrations were lower in cases of vaginal delivery ($b = 0.11$, SE 0.05, $p = 0.042$). Insulin levels were significantly elevated, as customized centiles increased ($b = 0.004$, SE = 0.002, $p = 0.016$). No significant correlation was found between insulin and nesfatin-1 in maternal and umbilical cord levels.

Conclusions: In this study nesfatin-1 levels are decreased in LGA compared to AGA fetuses. Fetal nesfatin-1 concentrations are higher in cases of GDM and cord blood nesfatin-1 concentrations are lower in cases of vaginal delivery.

1. Introduction

Impaired intrauterine growth has been implicated in abnormal glucose metabolism later in life [1]. Adults born LGA are at increased risk of metabolic syndrome, an entity comprising dyslipidemia, arterial hypertension, insulin resistance and type 2 diabetes mellitus (T2DM) [2]. The associated obesity reflects the imbalance between energy intake and expenditure [3]. In this respect, energy balance and satiety is regulated, not only by the hypothalamus, but also by the peripheral production of cytokines in the gut and adipose tissue [4]. Thus, adipose tissue has been recognized as an active endocrine organ implicated in insulin resistance [5] and satiety regulation [4,6].

Insulin is a potent regulator of fetal growth. Fetal hyperinsulinemia results in increased adipose tissue production and fetal overweight [7], linking to insulin resistance.

Nesfatin-1 is a newly identified 82 amino acid peptide derived from its larger precursor protein, nucleobindin2 (NUCB2) [8], that is encoded by the NUCB2 gene [8]. It regulates satiety/appetite and is abundantly expressed in several regions of the hypothalamus, that play key roles in controlling food intake [9]. When administered either centrally or peripherally, nesfatin-1 has been shown to reduce food intake in rodents [8,10]. Since nesfatin-1 crosses the blood–brain barrier [11], it is speculated that peripherally produced nesfatin-1 might influence appetite by acting on hypothalamic centers [10]. Nesfatin-1 is expressed in gastric endocrine cells [12], adipocytes [4,13] and β-islet cells [14].

Nesfatin-1 is a regulator of blood glucose levels, and endogenous nesfatin-1 is altered in diabetes [15]. Peripheral nesfatin-1 promotes release of insulin that regulates glucose and lipid metabolism, either directly on target organs, or via hypothalamic centers that control several metabolic pathways, like liver production of glucose [16]. Fasting plasma nesfatin-1 levels have been shown to be significantly lower in T2DM patients compared to healthy subjects [17]. Thus, nesfatin-1 is closely associated with glucose and insulin metabolism and notably insulin resistance [8,18].

In this study, we hypothesized that circulating nesfatin-1 and insulin levels should differ between LGA and AGA mother-infant

Abbreviations: AGA, appropriate for gestational age; GDM, gestational diabetes mellitus; LGA, large for gestational age; T2DM, type 2 diabetes mellitus; NUCB2, nucleobindin 2.

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pairs, since the former present with increased fat mass and undergo adaptational changes of endocrine/metabolic mechanisms, due to excessive intrauterine growth, possibly leading to insulin resistance. Therefore, we aimed to evaluate and correlate circulating nesfatin-1 and insulin concentrations in umbilical cord blood of LGA and AGA neonates and their mothers.

2. Materials and methods

The Ethics Committee of our teaching hospital approved the study protocol and all parturients provided signed informed consent before recruitment. All LGA infants (≥4000 g birth weight and >90 customized centile) born from July 2011 to December 2011 were consecutively collected and included in this study. For every two fullterm LGA infants, the first singleton, consecutively born fullterm AGA infant (birth weight between the 10th and 90th customized centile) was used as control. Thus, 40 LGA and 20 AGA infants, as well as their mothers comprised the cohort of this study. The Gestation Related Optimal Weight (GROW) computer-generated programme [19,20] was used to calculate the customized centile for each pregnancy. Significant determinants of birth weight, such as maternal height and booking weight, ethnic group, parity, gestational age and sex, were entered into the program to adjust the normal birth weight centile limits [19].

Gestational Diabetes Mellitus (GDM) was identified as the cause of LGA in nine out of the 40 pregnancies.

All neonates presented no symptoms of intrauterine infection or signs of genetic syndromes. One- and five-minute Apgar scores were ⩾8 in all LGA cases and AGA controls.

The demographic data of participating mothers and infants are listed in Table 1.

Mixed arteriovenous blood was collected from the doubly clamped umbilical cord reflecting fetal state and from the mother at the first stage of labor, or before elective cesarean section (called UC and MS, respectively). Blood was collected in pyrogen-free tubes and was immediately centrifuged after clotting. The supernatant serum was kept frozen at −80 °C until assay. The determination of nesfatin-1 levels was performed in the total 120 blood samples by Enzyme-Linked Immunosorbent Assay (Biovendor 62100 Brno, Chech Republic). The minimum detectable concentration, intra- and interassay coefficients of variation were 0.1 ng/ml (100 pg/ml), 2.5% and 3%, respectively.

In addition, serum insulin levels in all 120 samples were evaluated by Immuno Radiometric Assay (Immunotech s.r.o, 10227 Prague, Czech Republic). The minimum detectable concentration, intra- and interassay coefficients of variation were 0.5 μIU/ml, 3.4% and 4.3%, respectively.

3. Statistical analysis

Nesfatin-1 data (maternal and umbilical cord concentrations) did not follow normal distribution, thus they underwent logarithmic transformation. Linear regression analysis was used to estimate the effects of various parameters (maternal age, centile, birth-weight, mode of delivery, gestational age, gender, parity, GDM) on circulating nesfatin-1 levels.

Data regarding insulin did not follow normal distribution, thus, logarithmic transformation was applied. Linear regression analysis was used to estimate the effect of different factors (maternal age, centile, birth-weight, mode of delivery, gestational age, gender, parity, GDM) on insulin levels. Student's T test or Mann Whitney U test was used -where appropriate- to detect differences in quantitative variables between the two groups (LGA–AGA). Pearson's chi square test was used to estimate differences between categorical variables. Spearman correlation coefficient was used to examine any possible correlations between insulin and nesfatin-1. $P < 0.05$ was considered statistically significant.

4. Results

Median (ranges) for nesfatin-1 levels in AGA mothers and fetuses were 423.7 pg/ml (100–4413) and 204.4 pg/ml (100–2675), respectively, while for LGA mothers and fetuses were 405.3 pg/ml (100–5730) and 124.8 pg/ml (100–2332), respectively. Furthermore, median (ranges) for insulin levels in AGA mothers and fetuses were 6.9 μIU/ml (1.8–35.6) and 2.8 μIU/ml (0.5–16), respectively, while for LGA mothers and fetuses were 10.0 μIU/ml (0.5–92.3) and 4.0 μIU/ml (0.8–60.1), respectively (Figs. 1 and 2).

The effect of group, mode of delivery and GDM on nesfatin-1 UC levels was significant, after controlling for other confounding factors. Specifically, nesfatin-1 UC was significantly lower in LGA neonates as compared to AGA ($b = -0.206$, SE 0.07, $p = 0.005$). Moreover, nesfatin-1 UC was significantly elevated in neonates delivered by cesarean section ($b = 0.11$, SE 0.05, $p = 0.042$), as well as in those whose mothers had GDM after controlling for group ($b = 0.190$, SE 0.10, $p = 0.050$).

The effect of group, gender, birthweight, gestational age, mode of delivery, centile, maternal age, parity and GDM on insulin MS and UC levels were not significant. However, the effect of customized centile on insulin cord blood levels was proved significant; thus, insulin UC levels were significantly elevated as customized centile increased ($b = 0.004$, SE = 0.002, $p = 0.016$).

No significant positive or negative correlations were found between MS and UC insulin, as well as nesfatin-1 concentrations in

Table 1
Demographic data of participating mothers and their appropriate for gestational age (AGA) and large for gestational age (LGA) fetuses-neonates.

Variables	AGA [mean ± SD/median (range)]	LGA [mean ± SD/median (range)]	*P* value
Birth-weight (g)	3391 (3800–2800)	4215(4830–4000)	<0.001
Gestational age (weeks)	39.8 ± 0.7	39.3 ± 1.0	0.045
Centile	35 (71–12)	97 (100–90)	<0.001
Maternal age (years)	32 ± 4.2	31 ± 4.6	NS
Gender *N* (%)			NS
Male	10 (50)	27 (67.5)	
Female	10 (50)	13 (32.5)	
Mode of delivery *N* (%)			NS
Vaginal	13 (65)	18 (45)	
Caesarean	7 (35)	22 (55)	
Parity *N* (%)			NS
First	13 (65)	23 (57.5)	
Other	7 (35)	17 (42.5)	
Gestational diabetes mellitus *N* (%)			0.023
No	20 (100)	31 (77.5)	
Yes	0 (0)	9 (22.5)	