DESACYLATED GHRELIN AND LEPTIN IN THE CORD BLOOD OF SMALL-FOR-GESTATIONAL-AGE NEWBORNS WITH INTRAUTERINE GROWTH RESTRICTION

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Abstract

Context. Ghrelin, in both its acylated and desacylated forms, and leptin can modulate fetal energy balance and development.

Objective. The aim of our study is to assess desacylated ghrelin (DAG) and leptin values and influence on intrauterine and postnatal growth in infants with intrauterine growth restriction.

Design, subjects and methods. We performed a prospective study on 39 infants recruited over five months, 20 appropriate - for - gestational - age (AGA) infants and 19 small-for-gestational-age (SGA) infants, in which we measured DAG and leptin in the umbilical cord blood and we compared their respective values between the two groups, along with auxological parameters at birth and at 10 months of postnatal age.

Results. Our results show that both DAG and leptin have lower values in SGA infants and correlate with most of the anthropometrical parameters at birth. Both hormones correlate with weight at 10 months in SGA infants, but this correlation lacks in AGA infants. Whereas DAG in the cord blood can be considered a predictor for weight at 10 months ($\beta=0.207$, $p=0.001$), the same cannot be stated about leptin ($\beta=0.078$, $p=0.195$).

Conclusion. DAG and leptin are involved in both intrauterine and postnatal development, but the extent of their role is still to be determined.

Key words: desacylated ghrelin, leptin, infants, intrauterine growth restriction, small-for-gestational-age.

INTRODUCTION

Birth weight and length are expressions of intrauterine development, and thus represent essential clinical evaluation instruments in the neonatal period. These two parameters have always been used to identify the neonates at potential risk of presenting complications both in the neonatal period and

afterwards.

Intrauterine malnutrition represents the cause of low birth weight and length, it influences further childhood development and can potentially have consequences to adult health (1, 2). The failure of intrauterine development is influenced by a variety of hormonal factors, among which ghrelin and leptin are under particular scrutiny (3, 4). Both hormones have a major influence in regulating energy balance, but there is currently lack of available data regarding their role in intrauterine development (5-13).

On the one side, leptin is the hormone that influences long-term energy balance and nutrient intake, by diminishing food ingestion, and thus inducing weight loss; on the other side, ghrelin is a rapid acting hormone, with an important role in stimulating hunger and weight gain.

Ghrelin can be found in two circulating forms: acylated and desacylated, both with particular roles in regulating energy balance. For a long time, it has been believed that des-acylated ghrelin (DAG) is a metabolic by-product of acylated ghrelin. This statement was proved to be false according to recent research that demonstrated the independent, even antagonist, role of DAG (3,14,15). Although DAG has been found in fetal blood, available data regarding its role in fetal homeostasis are insufficient (5).

Leptin is secreted mainly by the adipose tissue, but also by the gastric endothelium and even the placenta (16, 17). Plasma leptin level is high in obese individuals and decreases with weight loss. Studies showed that fetal and placental leptins act individually, and placental leptin has no role in fetal development (6). Similarly to ghrelin, the role of leptin in fetal development is poorly understood (18).

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MATERIAL AND METHODS

We performed a prospective study over five months, between March 1st and July 31st, 2016 on a group of 39 infants admitted to the Neonatology Department of “Cuza-Vodă” Clinical Hospital of Obstetrics and Gynecology – 20 appropriate-for-gestational-age infants (scontrol group) and 19 small-for-gestational-age (SGA) infants with intrauterine growth restriction (IUGR), diagnosed by the Lubchenco Intrauterine Growth Curves (19). The infants were all born from mothers without known metabolic conditions, such as pregestational or gestational diabetes mellitus. We excluded all infants in which the IUGR was suspected to be due to genetic syndromes.

Venous cord blood sampled immediately after birth, in the delivery room or operating theatre, before initiation of enteral or parenteral nutrition, was analyzed through ELISA to determine desacylated ghrelin (DAG) and leptin.

In order to determine DAG, we sampled cord blood on EDTA, we obtained plasma by centrifugation over 15 minutes, at 4000 rot/min, and we used enzyme immunometric assay (EIA), after storing the samples for a maximum of 7 days at -20°C. We used Unacylated Ghrelin Human Standard and Unacylated Ghrelin Human Quality Control kits (BioVendor Research and Diagnostic Products, Brno, Czech Republic), with a minimum detection limit of 6 pg/mL. We compared the values in the study group to those of the control group (9-293 pg/mL).

For leptin determinations, we used the same biological product, stored the samples in the same conditions and we used Human Leptin ELISA Clinical Range kit (BioVendor Research and Diagnostic Products, Brno, Czech Republic), with a minimum detection limit of 0.2 ng/mL.

The analysis was performed using a

semiautomated UT-6500 microplate reader, fitted with a UT-3100-5 microplate washer (MRC Ltd., Holon, Israel).

The statistical analysis established the correlations between the anthropometrical data measured on the first day of life (weight, length, cranial circumference, body mass index, Rohrer ponderal index) and the levels of DAG and leptin. The coefficient for statistical significance was established at a value of $p \leq 0.05$.

RESULTS

All infants included in our study were term and late preterm infants, with gestational ages between 34 and 41 weeks (34-40 weeks in the control group, 35-41 weeks in the study group). The two groups were comparable regarding gestational age, but there were significant differences between the groups regarding anthropometric data (birth weight, birth length, cranial circumference, ponderal index and body mass index) (Table 1).

DAG was significantly lower in the study group (103 pg/mL $\pm$ 130.13 SD), compared to the control group (115.58 pg/mL $\pm$ 83.89 SD) ($p=0.0003$). In the study group, DAG values varied between 0 and 473.9 pg/mL, and 50% of the infants had values below 47.51 pg/mL (Table 1, Fig. 1).

DAG correlated directly with birth weight ($p=0.001$), cranial circumference ($p<0.001$), thoracic circumference ($p<0.001$), ponderal index ($p<0.001$) and body mass index ($p<0.001$). In the group of SGA infants with IUGR, DAG did not correlate with birth length ($p=0.075$), but the correlation exists in the control group ($p=0.0091$). DAG values did not correlate with gestational age ($p=0.087$) (Table 2, Fig. 1).

Leptin values in the study group (4.45 ng/mL $\pm$ 13.87 SD) were significantly lower ($p<0.001$)

Table 1. Anthropometric and biochemical characteristics of infants in the studied groups

Clinical parameters	Study group [†] Mean $\pm$ SD	Control group [†] Mean $\pm$ SD	H _{Kruskal-Wallis}	p(95% CI)
Gestational age (weeks)	39.2 $\pm$ 1.5	38.2 $\pm$ 1.5	3.827	0.058
Weight (g)	2594.7 $\pm$ 358	3497.5 $\pm$ 403.6	23.984	<0.001*
Length (cm)	47.6 $\pm$ 1.5	51.9 $\pm$ 2.1	22.977	<0.001*
Birth Head circumference (cm)	32.6 $\pm$ 1.6	34.4 $\pm$ 1.1	12.521	0.0004*
Body mass index (kg/m ²)	11.4 $\pm$ 1.1	12.9 $\pm$ 0.7	19.471	0.00001*
Ponderal index (kg/m ³)	2.4 $\pm$ 0.2	2.5 $\pm$ 0.1	3.982	0.0046*
Desacylated ghrelin (pg/mL)	103 $\pm$ 130.13	118.58 $\pm$ 83.89	13.232	0.0003*
Leptin (ng/mL)	4.45 $\pm$ 3.87	9.32 $\pm$ 10.22	60.799	<0.001*
Weight at 10 months (kg)	9.06 $\pm$ 1.18	9.54 $\pm$ 1.20	7.537	

[†] Continuous variables were expressed as: mean $\pm$ standard deviation; categorical variables: number (%);

[‡] Kruskal-Wallis; Chi-square test or Fisher's exact test; (*) Marked effects are significant at $p < 0.05$.

Figure 1. Correlation of DAG with birth weight and length.

Figure 2. Correlation of leptin with birth weight and length.

Table 2. Correlations of DAG/leptin values and anthropometric parameters

Anthropometric parameters			Study group		Control group	
vs. desacetylated ghrelin	$r^{\dagger}$ (n=39)	95% CI p	$r^{\ddagger}$ (n=19)	p	$r^{\ddagger}$ (n=20)	p
Gestational age	0.1040	0.087	0.1460	0.073	0.0657	0.476
Weight	0.2069	0.001	0.2339	0.0037	0.3529	0.00008
Length	0.1491	0.014	0.1444	0.0759	0.2371	0.0091
Birth Head circumference	0.4116	<0.001	0.4584	<0.01	0.4439	<0.001
Body mass index	0.2614	<0.001	0.2673	0.001	0.3980	<0.001
Ponderal index	0.2755	<0.001	0.2676	0.001	0.2882	0.001
Weight at 10 months	0.3143	<0.001	0.3174	<0.01	0.1766	0.062
vs. leptin						
Gestational age	0.2356	<0.001	0.2803	<0.001	0.4472	<0.001
Weight	0.4207	<0.001	0.2734	0.0007	0.2973	0.0002
Length	0.3977	<0.001	0.0717	0.3799	0.3707	<0.001
Birth Head circumference	0.217	0.190	0.0592	0.469	0.0550	0.501
Body mass index	0.4044	<0.001	0.3740	<0.01	0.1530	0.060
Ponderal index	0.2868	<0.001	0.4208	<0.01	-0.1538	0.059
Weight at 10 months	0.2946	0.0109	0.4515	0.0242	0.0946	0.109

† Pearson Correlation ; ‡ Spearman Rank Correlation.

Table 3. Calculated coefficients in multiple linear regression

10 month weight	Unstandardized Coefficients		Standardized Coefficients	t	Sig.p
	β	Std. Error	Beta		
Intercept			46.52229	4.73903	0.000004
Ghrelin (pg/mL)	0.207282	0.062834	0.00223	3.29890	0.001116
Leptin (ng/mL)	0.078198	0.060267	0.02188	1.29752	0.195677

R= .37395448 R²= .13984195 Adjusted R²= .12926624; F(3,32)=13.223 p<.00000 Std.Error of estimate: 1.1142.

compared to the control group (9.32 ng/mL $\pm$ 10.22 SD). In SGA infants with IUGR, leptin values were between 0.66 and 15.03 ng/mL, and 50% of the cases had values lower than 3.04 ng/mL (Table 1, Fig. 2).

Leptin values correlated with gestational age ($p<0.001$, $r=0.2356$), birth weight ($p=0.0002$, $r=0.2973$) and birth length ($p<0.0001$, $r=0.3977$). Also, there was a significant correlation of leptin with the body mass index ($p<0.01$, $r=0.374$) and ponderal index ($p<0.01$, $r=0.4208$) in the study group, but not in the control group (Table 2).

The results of the multivariate analysis show that high values of DAG correlated with higher body weight at the age of 10 months ($\beta=0.207$, $p=0.001$), and leptin did not represent a significant predictor for weight at the same age ($\beta=0.078$, $p=0.195$) (Table 3).

DISCUSSION

The main role of ghrelin is to positively regulate the energy balance. Total ghrelin can be found in two circulating forms – acylated and desacylated ghrelin (DAG), with different roles in metabolic homeostasis. DAG has higher serum levels and was shown to downregulate nutrient intake (15). Also, high levels of DAG have been documented in adult patients with anorexia nervosa (20).

Ghrelin is mainly produced in the stomach, but also in the placenta, suggesting an important role in intrauterine energy balance. Studies have found that cord total ghrelin levels increase with increasing gestational age (21), but remain constant throughout late gestation (22). Total ghrelin does not correlate with birth weight, gestational age, BMI, birth length or head circumference in appropriate-for-gestational age infants of healthy mothers (5). However, concentrations of total ghrelin in the cord blood of SGA infants are significantly higher than in appropriate-for-gestational age or large-for-gestational age at different times of gestation (13, 23, 24). Moreover, it has been speculated that SGA infants with lower ghrelin levels in the cord blood have slow postnatal weight gain (25).

Studies quantifying acylated ghrelin have

not found any correlation with birth weight or other anthropometrical or biochemical parameters in term neonates (26). The opposite is apparently true for DAG: Gonzalez-Dominguez *et al.* showed that DAG concentrations in the cord blood are significantly higher in SGA infants compared to AGA newborns, and this parameter showed a negative correlation with birth weight (8). By contrast, our results showed a significantly lower concentration of DAG in the cord blood of SGA infants with IUGR and a direct positive correlation with birth weight and other auxological parameters. Similarly, lower serum DAG have been found in the blood of obese subjects (27–29), indicating a potential role of the hormone in the development of the "thrifty phenotype" described by Barker (30, 31). These contrasting results could be explained by the small data sets, by different methods of analysis and also by the insufficient knowledge to date about the influence of DAG in intrauterine malnutrition.

Ghrelin values change significantly during the next stages of development, with significant correlation to anthropometric growth and the particular needs of extrauterine life. The highest values of total ghrelin have been registered in the early postnatal period, until the growth hormone begins its regulatory function of nutrient intake and growth, so the role of total ghrelin is similar to the growth hormone (8, 32–36). Later, total ghrelin maintains high values in infants with normal weight and is lower in those with obesity or accelerated growth during the first year of life (37, 38).

In our study, DAG values in the cord blood positively correlate with infant weight at the age of 10 months in SGA infants and could be considered prediction factors for weight at this age. Mean DAG values in the study group are significantly lower than in the control group and can be both a cause and a consequence of IUGR. Our study is, to our knowledge, the first to correlate DAG values from this cord blood with later anthropometrical growth. The limits of our research are the lack of total ghrelin and acylated ghrelin values in neonates, maternal determinations and later determinations in infants to create a more complex hormonal profile. Even so, there is currently a marked

lack of data regarding the role of DAG and acylated ghrelin in potentially regulated long-term metabolic balance.

In physiological conditions, leptin is an essential component of energy balance. Leptin acts mainly in the hypothalamus, by inhibiting hunger and initiating catabolism. On the peripheral level, leptin inhibits fat accumulation, thus maintaining glucose sensitivity. It acts as a regulator of fatty tissue, which means that an optimum level of leptin throughout development leads to optimal growth and maturation of the main metabolic regulation pathways. Even short periods of hypo- or hyperleptinemia lead to metabolic disturbances and the initiation of compensatory mechanisms (10). Animal studies showed that there is a high postnatal level of leptin and all throughout breastfeeding which, by contrast with adults, does not inhibit hunger (39), but acts as a neurotrophic agent, by stimulating cerebral growth and development (40, 41). High leptin values in macrosomic newborns and low values in infants with IUGR seem to be a consequence, rather than a determinant of fetal growth. This hypothesis is supported by the finding that newborns with total congenital leptin deficit seem to have normal birth weight (42), so it is still debatable whether or not leptin can be considered an intrauterine growth factor. Leptin has a positive correlation with birth weight, and thus has low values in SGA infants (6, 43, 44). Our study supports this finding: mean leptin values were lower in infants with IUGR – 4.45 ng/mL (0.66–15.03 ng/mL), *versus* 9.32 ng/mL (1.73–50 ng/mL) in infants in the control group. Leptin values correlate with anthropometrical parameters in both groups, but the correlation is stronger in SGA infants.

The correlation of leptin with gestational age may be explained by leptin production by the intra-abdominal fatty tissue, which is more developed in IUGR term infants and is directly responsible for the development of adult metabolic conditions.

Similarly to the results of Mantzaros *et al.* (45), low leptin values in the umbilical cord blood correlate with low birth weight, but later accelerated weight gain. Unlike their study, where it was found that leptin levels in the umbilical cord blood can be considered predictive factors for later weight gain, the multivariate analysis we present does not support this claim at the postnatal age of 10 months.

Both ghrelin/DAG and leptin values could be influenced on the long term by a number of different other factors, such as early postnatal feeding (moment of starting enteral feeds and tolerance of said feeds,

exclusive breastfeeding, mixed diets, formula feeding and type of formula) or complementary feeding (including moment of start and composition), but these parameters are too diverse to be standardized and applied to our small group of infants.

The multivariate analysis demonstrated that DAG values, and not leptin, represent a prediction factor for weight at 10 months of age.

The relationship between DAG and leptin on the one side, and IUGR on the other side, is not fully understood, and the same can be stated about the role of these two hormones in fetal and postnatal development. Data about the placenta, maternal and postnatal values of DAG and leptin are required for further knowledge, as are growth parameters and postnatal nutrition.

In conclusion, the results of our study demonstrate that DAG and leptin have synergic roles and their values determined in the umbilical cord blood significantly correlate with anthropometrical parameters. This correlation is stronger in infants with IUGR.

The data we obtained is significant in terms of the understanding of fetal and neonatal metabolism and could represent a step further in the research regarding fetal programming and its influence on subsequent infant and child growth and development.

Conflict of interest

The authors declare that they have no conflict of interest.

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