Association of Cord Blood Des-Acyl Ghrelin with Apgar Score and Anthropometric Measures in Relation to Its Maternal One

Enas R. Abdelhamid¹, Alyaa H. Kamhawy¹*, Hanaa H. Ahmed², Moines M. Abu Shady¹, Ahmed Fathy¹ and Reham F. Fahmy¹

¹Department of Childhealth, Medical Division, National Research Center (NRC), Cairo, Egypt, 33rd El Bohouth st, Former El Tahrir st, Dokki, Giza, POB:12311, Egypt.
²Department of Hormones, Medical Division, National Research Center (NRC), Cairo, Egypt, 33rd El Bohouth st, former El Tahrir st, Dokki, Giza, POB:12311, Egypt.
*Corresponding author E-mail: alyaakamhawy@hotmail.com

http://dx.doi.org/10.13005/bpj/1743

(Received: 07 May 2019; accepted: 20 July 2019)

Ghrelin is a pleiotropic hormone that governs eating and energy equilibrium as well as promotes the release of growth hormone release. Although cord blood ghrelin has been correlated to the weight at birth and other anthropometric measures, the implication of des-acyl ghrelin in fetal and postnatal growth still not well understood. The goal of this research was to investigate the concentration of des-acyl ghrelin (DAG) in cord blood of appropriate for gestational age (AGA), small for gestational age (SGA) and large for gestational age (LGA) infants in order to elucidate their correlation with birth weight, anthropometric measures as well as apgar score of the newborn and relating them to maternal DAG levels. Cord blood specimens were procured from 25 SGA, 24 AGA infants and 25 LGA infants. Desacyl ghrelin, was quantified by ELIZA. A significant negative correlation has been found between cord ghrelin and neonatal anthropometric measures (neonatal weight, height, head circumference, mean arm circumference) as well, a significant negative correlation has been detected between cord ghrelin and apgar score at 1 and 5 min. The current results shed the light on the critical role of DAG in the growth during the intrauterine period. Further studies are necessary to identify the exact mechanisms underlying the contribution of DAG in the growth of the fetus.

Keywords: Apgar Score; Anthropometric Measures; Birth Weight; Cord Blood; Des-Acyl Ghrelin.

Birth weight may have a strong insult on the metabolic health throughout the adulthood. When born either SGA or LGA may strength the risks for metabolic syndrome, obesity, T2DM, and CVD in adult life. It has been reported that about 10% of newborn babies in the US have birth weight aberrations either SGA or LGA. The onset of macrosomia and LGA has been reported to be enhanced over decennium in numerous nations. LGA is correlated with increased risks of neonatal morbidity, neonatal and maternal harm as well as Caesarean section delivery. The factors affecting birth weight include genetics, gestational diabetes, duration of gestation, and ethnic factors. Preceding reports mentioned that cord blood ghrelin levels have a negative correlation with body weight in SGA. Meanwhile, the role of cord blood ghrelin levels in LGA is not well approved till now. Therefore, the establishment of birth weight and factors affecting perinatal development...
represent an important issue in avoiding metabolic illness, which constitute a major public health concern globally. Many factors like nutrients and hormones among them the ghrelin hormone has been proposed to have a function in fetal growth and participate in birth weight establishment\(^\text{12,13}\).

Firstly, ghrelin was recognized as the endogenous ligand for growth hormone secretagogue receptor (GHS-R1)\(^\text{14}\). Ghrelin (a 28-amino-acid peptide) is basically liberated from the cells of gastric mucosa and has a critical act in the regulation of appetite and energy equilibrium. It was discovered via its influence on GH release, and thus it was suggested to be influential in growth\(^\text{15,16}\). This hormone may present in circulation in two molecular types, des-acyl ghrelin (DAG), and acyl ghrelin (AG), in which serine 3 residue is n-octanoylacylated. DAG is hypothesized to be a disintegration output of AG with no physiological function\(^\text{14}\). Meanwhile, recently it has been proved that DAG is an active hormone with many impacts on various tissues in numerous physiological and pathophysiological conditions\(^\text{17}\).

Several other studies have also been related to ghrelin and its effect in growth\[^{18,19}\]. Apart from its action on GH release, ghrelin has been found to increase appetite and initiate adiposity\(^\text{20}\). Infant with SGA newborns experienced an inhibited rate of development during the intrauterine interval, which might be attributed to fetal, maternal or environmental factors. These infants have a decreased concentration of GH after delivery as a result of nutritional shortage before birth\(^\text{16}\).

In spite of most of the neonates may catch up growth soon after birth, different manners of weight gain are noticed in infancy, which could be linked to feeding and fasting\[^{21,19}\]. A solid association has been recorded between very low birth weight and infant mortality, which may be owing to excessive fetal growth regression\[^{22}\]. Considering the essential association between feeding and development and the orexigenic action of ghrelin, this hormone appears to be strongly related to postnatal growth.

Existence of ghrelin in the cord blood has been detected but little reports have quantified ghrelin levels in the umbilical cord of newborn infants\[^{25,10}\].

In adult humans, it has been observed that plasma ghrelin is up-regulated prior each of the three main meals and down-regulated to a nadir 90 min post meal, proposing that it has a physiological role in meal regulation\[^{24}\]. It was suggested that oxyntic gland cells of the stomach contain food-entrainable oscillators that induce signal of timed ghrelin liberation that influences both brain and peripheral spots\[^{25}\].

Ghrelin is well linked with glucose metabolism and body mass. Broglio et al. mentioned that, ghrelin promotes hyperglycemia and lowers plasma insulin levels\[^{26}\]. On the opposite side plasma ghrelin concentrations increased markedly in Prader-Willi syndrome patients, a genetic disorder characterized by insatiable appetite and massive obesity\[^{27}\] indicating that the overproduction of ghrelin may be accountable for the food-seeking behavior in these patients.

Ghrelin has formerly been revealed to be enhanced in anorexia and children with poor appetite\[^{28}\] and decreased in obesity, having a role in the governing of energy balance; thus, it was proposed that ghrelin constitutes the chronic nutritional condition\[^{29,11}\].

In this current investigation, we goaled to analyze the levels of DAG, in umbilical cord blood of SGA, AGA, and LGA newborns; and its interconnection with weight at birth, in order to evaluate their role in birth weight establishment, anthropometric measures of the newborn as well as apgar score of the newborn and relating them to maternal DAG levels.

Subjects and methods

Study design and target population

We carried a descriptive, comparative, and transversal study in healthy women with singleton pregnancy, recruited from el Galaa Hospital from January 2017 to August 2017. Written informed consent was taken in advance from the mother of each newborn. The study had the ethical approval of the medical research committee at the NRC having number 18/068.

Inclusion criteria included women between 18 and 35 years of age and their full-term babies from normal delivery or cesarean section, without any birth complications including perinatal asphyxia, or acute fetal suffering signs were included in this research. Exclusion criteria included women presenting with diabetes, preeclampsia,
antiphospholipid syndrome, connective tissue diseases, chronic infection, alcoholism, or smoking during the pregnancy.

Newborns were assigned in accordance to their birth weight in SGA, (lowest 10th percentile), AGA (between 10th and 90th percentile), and LGA (higher 90th percentile). We collected clinical and anthropometrical information from mothers and newborns by direct interview and clinical sheets. Maternal blood samples were withdrawn during labor after being fasting for 3–8 h. Umbilical cord blood was gathered immediately after delivery. Serum was obtained by centrifugation at 4°C at 1800 xg within 1 h after being collected, aliquoted and stored at -20°C until analysis.

Total ghrelin levels (ng mL⁻¹) were measured by commercial enzyme-linked immunosorbent assay (ELISA) kit (Phoenix Pharmaceuticals, Belmont, CA, USA).

**Statistical analysis**

Data management and analysis were carried out using the Statistical Package for Social Sciences (SPSS) vs. 21. Numerical data were summarized using means and standard deviations or medians and ranges. Categorical data were summarized as percentages. Comparisons between groups for normally distributed numeric variables were done using the Student’s t-test while for non-normally distributed numeric variables were performed by Mann-Whitney test. Chi square test or Fisher’s exact test were applied to compare between the groups with respect to categorical data. To measure the strength of association between numeric variables, Spearman’s correlation coefficients were computed. All p-values are two-sided. Statistically significant P-value was set at < 0.05.

**RESULTS**

Table 1 shows a significant difference between SGA, AGA, and LGA as regarding weight, length, head circumference and mean arm circumference.

<table>
<thead>
<tr>
<th>Variable</th>
<th>SGA mean± SD</th>
<th>AGA mean± SD</th>
<th>LGA mean± SD</th>
<th>F-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mother Age</td>
<td>26.13±5.80</td>
<td>25.76±4.89</td>
<td>26.38±5.32</td>
<td>0.064</td>
<td>0.938</td>
</tr>
<tr>
<td>Mother weight</td>
<td>71.92±16.90</td>
<td>76.62±15.847</td>
<td>84.97±9.343</td>
<td>3.104</td>
<td>0.052</td>
</tr>
<tr>
<td>Mother height</td>
<td>15.72±5.02</td>
<td>15.81±6.95</td>
<td>16.54±7.33</td>
<td>1.139</td>
<td>0.327</td>
</tr>
<tr>
<td>Neonatal weight</td>
<td>1.96±0.356</td>
<td>3.053±0.2167</td>
<td>4.115±0.4518</td>
<td>138.713</td>
<td>0.000*</td>
</tr>
<tr>
<td>Neonatal length</td>
<td>43.21±3.12</td>
<td>43.21±3.12</td>
<td>51.15±2.15</td>
<td>53.591</td>
<td>0.000*</td>
</tr>
<tr>
<td>HC</td>
<td>31.71±1.71</td>
<td>34.84±1.07</td>
<td>36.15±1.34</td>
<td>51.724</td>
<td>0.000*</td>
</tr>
<tr>
<td>MAC</td>
<td>8.60±1.103</td>
<td>10.36±0.941</td>
<td>12.00±1.000</td>
<td>49.110</td>
<td>0.000*</td>
</tr>
<tr>
<td>BMI</td>
<td>28.99±6.2190</td>
<td>30.63±5.8594</td>
<td>33.11±4.2766</td>
<td>2.195</td>
<td>0.120</td>
</tr>
</tbody>
</table>

**Table 2.** Clinical and laboratory features of SGA, AGA, and LGA newborns.

<table>
<thead>
<tr>
<th>Variable</th>
<th>SGA mean± SD</th>
<th>AGA mean± SD</th>
<th>LGA mean± SD</th>
<th>F-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age</td>
<td>37.83±1.52</td>
<td>37.68±0.95</td>
<td>37.00±1.73</td>
<td>1.637</td>
<td>0.203</td>
</tr>
<tr>
<td>Apgar score at the first minute</td>
<td>4.64±1.15</td>
<td>6.24±1.51</td>
<td>5.00±1.49</td>
<td>6.115</td>
<td>0.005*</td>
</tr>
<tr>
<td>Apgar score at the 5th minute</td>
<td>6.79±1.19</td>
<td>8.57±1.25</td>
<td>8.10±1.29</td>
<td>8.884</td>
<td>0.001*</td>
</tr>
<tr>
<td>Maternal des-acyl ghrelin (pg/ml)</td>
<td>731.30±235.47</td>
<td>627.50±231.25</td>
<td>684.62±149.70</td>
<td>0.744</td>
<td>0.482</td>
</tr>
<tr>
<td>Cord blood des-acyl ghrelin (pg/ml)</td>
<td>662.61</td>
<td>500.00</td>
<td>524.62</td>
<td>4.854</td>
<td>0.013*</td>
</tr>
</tbody>
</table>

P < 0.05 is significant
Table (2) shows a significant difference between SGA, AGA, and LGA as regarding Apgar score at the 1st and 5th minute and level of cord blood ghrelin.

The results in table (3) indicated that Neonatal weight, gestational age, and Apgar score at the first minute are considered to be the main predictors of cord blood des-acyl ghrelin. The low baby weight and low Apgar score at the first minute are associated with high cord blood des-acyl ghrelin.

The data in table (4) shows that there is a significant negative correlation between cord blood des-acyl ghrelin and neonatal anthropometric measures (neonatal weight, height, head circumference, mean arm circumference) also. There is a significant negative correlation between cord blood des-acyl ghrelin and Apgar score at 1 and 5 min.

### Table 3. Multiple linear regression analysis of the linkage between cord ghrelin levels and maternal and neonatal features

<table>
<thead>
<tr>
<th></th>
<th>Unstandardized Coefficients</th>
<th>Standardized Coefficients</th>
<th>t</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Constant)</td>
<td>5713.755</td>
<td>4170.705</td>
<td>1.370</td>
<td>.188</td>
</tr>
<tr>
<td>Mother weight</td>
<td>32.376</td>
<td>24.185</td>
<td>1.339</td>
<td>.197</td>
</tr>
<tr>
<td>Mother height</td>
<td>-23.389</td>
<td>24.742</td>
<td>-.945</td>
<td>.357</td>
</tr>
<tr>
<td>Neonatal weight</td>
<td>-210.470</td>
<td>67.153</td>
<td>-3.134</td>
<td>.006*</td>
</tr>
<tr>
<td>Neonatal length</td>
<td>41.137</td>
<td>22.886</td>
<td>1.797</td>
<td>.089</td>
</tr>
<tr>
<td>HC</td>
<td>5.020</td>
<td>28.990</td>
<td>1.001</td>
<td>.330</td>
</tr>
<tr>
<td>MAC</td>
<td>-49.806</td>
<td>36.843</td>
<td>-2.723</td>
<td>.014*</td>
</tr>
<tr>
<td>Apgar score at the first minute</td>
<td>-92.270</td>
<td>33.879</td>
<td>2.632</td>
<td>.017*</td>
</tr>
<tr>
<td>Apgar score at the 5th minute</td>
<td>35.623</td>
<td>35.570</td>
<td>1.001</td>
<td>.330</td>
</tr>
<tr>
<td>Gestational age</td>
<td>-47.894</td>
<td>18.198</td>
<td>-2.632</td>
<td>.017*</td>
</tr>
<tr>
<td>Maternal des-acyl ghrelin (pg/ml)</td>
<td>-4.992</td>
<td>.111</td>
<td>-2.448</td>
<td>.060</td>
</tr>
</tbody>
</table>

### Table 4. Correlation between cord ghrelin and neonatal variables

<table>
<thead>
<tr>
<th></th>
<th>Neonatal weight</th>
<th>Neonatal length</th>
<th>HC</th>
<th>MAC</th>
<th>Apgar score at the first minute</th>
<th>Apgar score at the 5th minute</th>
<th>Ghrelin in mother (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cord blood</td>
<td>-.479(***), p  .001</td>
<td>-.376(*), p .012</td>
<td>-.480(**), p .001</td>
<td>-.412(**), p .005</td>
<td>-.359(*), p .047</td>
<td>-.412(*), p .021</td>
<td>-.095</td>
</tr>
<tr>
<td>des-acyl ghrelin</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

** Correlation is significant at the 0.01 level (2-tailed).
* Correlation is significant at the 0.05 level (2-tailed).

### DISCUSSION

Intrauterine growth is a complicated vital process, in which placental development and maternal status have a critical role. In this research work, we observed that cord blood DAG recorded higher levels in SGA than AGA and LGA newborns and thus it is being correlated with birth weight. This finding agrees with that of Martha et al. who found that cord blood DAG levels of SGA are significantly higher comparative to those of AGA, and also these investigators observed that DAG is correlated negatively with birth weight. This indicates that DAG may have a prominent role in birth weight initiation [30].

Our result also comes in line with that of Dingel et al. who mentioned that the cord blood des-acyl ghrelin concentration of LGA are much lower when compared to AGA and SGA and its levels...
have inverse correlation with birth weight[31]. Meanwhile, the inverse association between circulating des-acyl ghrelin and body weight in LGA still not observed by other studies[31,32].

The current study revealed negative association between cord blood des-acyl ghrelin concentrations and gestational age as detected by[10,11,13]. This may suggest that SGA infants usually exhibit a quick postnatal growth and weight gain higher than their AGA and LGA counterparts, a situation called catch-up growth. A growing body of evidence has linked this status to a high risk of adipose tissue deposition, IR, and CVD in adulthood [33]. It has been suggested that umbilical cord total ghrelin may be a powerful indicator for postnatal catch-up growth in SGA newborns. Other researches found that high total ghrelin concentrations in SGA neonates at birth stay up-regulated till 3 months of age, correlating with anthropometrical indices at birth and early postnatal growth[34]. In discordant growth monozygotic twins, SGA twins with a more ghrelin concentrations has a higher prognosis for catch-up growth[35]. On the opposite hand, other studies reported that fasting total ghrelin levels have no difference in AGA and SGA infants early in life tell first year[23]. Other more recent studies have demonstrated that DAG blood concentrations in infants one week of age born SGA are greater than their AGA infants, and DAG is negatively correlated with birth weight[36]. This may suggest that DAG contributes in early postnatal growth in SGA, probably as a sign for early aggregation of lipids and intake programming of energy and the function of DAG as an adaptive indicator in SGA that may occur during fetal and early postnatal development.

In the present study there is a no association between cord blood des-acyl ghrelin concentrations and maternal des-acyl ghrelin levels. This result disagree with Ding et al. who found that there is a positive association between cord blood ghrelin concentrations and maternal ghrelin levels[31]. This might be attributed to a relative small sample size or healthy status of the mothers that were examined. In this work, mothers with any metabolic disorders like high blood pressure, diabetes and any other metabolic illness were not included in the study.

Previous studies suggested that maternal total ghrelin levels in pregnancy maximize at midgestation and diminish toward term, conformable with the evolution of insulin resistance[37]. In contrast, in the second and third trimester, ghrelin levels elevate in maternal blood and correlate positively with waisttof the neonate indicating a potent role of ghrelin in maternal energy control, probably supporting nutrient supply to the fetus.

In this study we found a significant association between cord blood des-acyl ghrelin and anthropometric measures of the baby(baby weight, height, head circumference and mean arm circumference) and this goes hand in hand with Ding et al. who stated that cord blood ghrelin levels and body length, head circumference and BMI were negatively correlated[31]. Other reports have not observed any correlation with birth weight or other biochemical or anthropometrical parameters in term of neonates[39,40]. Also DAG levels in AGA and LGA infants showed no significant change in our study but it was negatively correlated with birth weight in SGA. These findings are in harmony with those obtained from the other studies evaluating total ghrelin in cord blood, where an inverse correlation with birth weight[11,13] and head circumference[32] has been observed, with no alterations in total ghrelin levels in LGA.

In the contrary to SGA catch-up growth, LGA and AGA infants usually preserve their growth and development ratio during postnatal life from one year and up to the age of 4 years[41]. Moreover, LGA newborns constitute near 40% rate of obesity[42], thus elevating the risk of metabolic disorders in future life. A drop in DAG and total ghrelin have been recorded both in metabolically abnormal children and healthy obese versus their lean subjects, indicating a role for ghrelin isoforms in insulin resistance and adipose tissue formation since childhood[43].

Furthermore, total ghrelin concentrations in prepuberal non-obese children born LGA are blunted relative to BMI-matched normal children born AGA[44]. In accordance with our findings, this findings favorsthefunction of neonatal DAG as a metabolic programming sign for postnatal and perhaps long-term adjustment of body weight and energy homeostasis in LGA neonate.

In the present study, using multiple regression analysis baby weight, gestational age and apgar score at the first minute have been
considered as the main predictors of baby ghrelin. These match with Ding et al. who mentioned that the body weight is the essential actor correlated with cord ghrelin levels. In our study, the level of des-acyl ghrelin doesn’t differ between both genders, and this agrees with Bellone et al. (2012) who cited that both forms of ghrelin are independent of gender. The type of delivery does not affect ghrelin levels in our study nor in the studies done by others.

In the current study we recorded a significant positive association between DAG and apgar score at 1 min but this doesn’t agree with Pak et al. who didn’t find any correlation with apgar score at 1 or 5 min.

This observations of an inverse relationship between ghrelin and apgar score and anthropometric indices indicated that ghrelin may adopt its active physiological function in adapting growth and energy homeostasis at late stages of gestation (e.g. 37 weeks). This suggestion may be effective to term newborns, as ghrelin may help in triggering appetite and preserving an enough blood glucose level when energy and nutritional supplies from the mother are interrupted after birth. Although all the above mentioned studies indicated that cord blood ghrelin levels of SGA infants are higher comparative to that of AGA newborn, and proposed that ghrelin is influenced by nutritional condition during the intrauterine life, in our present study we didn’t find any correlation between maternal nutritional state and cord blood des-acyl ghrelin.

In conclusion, the outcomes of the present study suggest that des-acyl ghrelin level is an indicator for the body’s nutritional status and DAG may play a key role as a metabolic sign for adaptation of energy homeostasis and perinatal growth in early life. Further studies are necessary in order to justify the effect of maternal environment on DAG, the potent role of DAG as a long term governor of metabolic status, and to better underlying the mechanisms through which DAG contributes in intrauterine and neonatal growth.

ACKNOWLEDGEMENT:

We thank the National Research Centre (in-house office for research projects) for the research grants supported this work. Furthermore, we thank Al Gallaa Hospital for their help and assistance.

REFERENCES


