Umbilical Cord Ghrelin in Term and Preterm Newborns and its Relation to Metabolic Hormones and Anthropometric Measurements

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Abstract: The aim of the study was to assess umbilical cord ghrelin level in term and preterm newborns and its relation to other metabolic hormones and anthropometric measurements. A cross sectional comparative study included 50 normal appropriate-for-gestational-age newborns (25 full-terms; 25 preterm). Assessment of anthropometric measurements, cord levels of ghrelin, leptin, insulin and glucose were done to all newborns. Umbilical cord ghrelin was detected in all newborns. There was no significant difference between term and preterm groups regarding ghrelin, insulin and glucose. Leptin was significantly lower in preterm than term group. Sex and mode of delivery had no effects regarding all studied variables. There was no overall correlation between ghrelin and gestational age, anthropometric measurements, leptin, insulin or glucose in all newborns. Preterm group demonstrated significant correlations between ghrelin and weight, body mass index and abdominal circumference. An overall significant correlation was found between leptin and gestational age and anthropometric measurements in all newborns. In preterm group leptin correlated with weight, length, subscapular skin-fold thickness and abdominal circumference. To conclude the umbilical cord ghrelin was relatively invariable at birth between 30 and 41 weeks gestation showing no gestational age-related variation, unlike leptin, which was lower in preterm group indicating increased adipose mass and placental maturation with increased gestational age.

Key words: Ghrelin, full-term newborns, preterm newborns, metabolic hormones, anthropometric measurements

INTRODUCTION

Ghrelin and leptin are peripheral hormones, together with insulin and glucocorticoids, which permit the central regulation of energy balance. These peripheral hormones exert their effects on energy homeostasis either by activating or inhibiting the activity of orexigenic or anorexigenic peptides within the hypothalamus (Sainsbury et al., 2002; Toshinai et al., 2003). They play an important role in the regulation of food intake and body weight (Klok et al., 2007).

Ghrelin is a 28 amino-acid peptide, produced predominantly by the stomach and acts as an endogenous ligand for Growth Hormone Secretagogue Receptor (GHS-R) (Kojima et al., 1999). Its levels vary from fetal life through early adulthood (Soriano-Guillén et al., 2004). The highest levels of ghrelin are found during early postnatal life, when growth hormone begins to exert its effects on growth and important changes in food intake occur (Kitamura et al., 2003). It has also been detected in cord blood (Chaconne et al., 2002). Ghrelin increases body weight and growth hormone secretion and produce positive energy balance, decrease energy expenditure and increase fat storage (Nakazato et al., 2001; Druce et al., 2005; Schmid et al., 2005). It regulates gastric motility and attenuates reduction in food intake and body weight induced by leptin since it acts as an antagonist of leptin through hypothalamic nuclei (Toshinai et al., 2003; Nakazato et al., 2001; Brogio et al., 2001; Murray et al., 2005). The physiological role of ghrelin in newborn babies is not clear (Fuglsang et al., 2006).

Leptin is a 167 amino-acid peptide encoded by the obesity gene (Bray and York, 1997; Zhang et al., 1994). It is secreted by fat tissue and suppresses food intake and increases energy expenditure (Rohner-Jeanrenaud and Jeanrenaud, 1996). It binds to leptin receptors in the hypothalamus, encoding orexigenic and anorexigenic neuropeptides (Sahu, 2003). Leptin levels are influenced by the amount of body fat, since they are found high in obese and low in lean individuals (Monteleone et al., 2002). Leptin concentration changes during fetal and neonatal periods (Matsuda et al., 1999). A relatively high level of leptin at birth and the expression of leptin in the

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placenta suggested that leptin may play a role during the perinatal period (Matsuda et al., 1999; Masuzaki et al., 1997).

The aim of this study was to assess umbilical cord ghrelin level in term and preterm newborns and its relation to other metabolic hormones and anthropometric measurements.

**MATERIALS AND METHODS**

This study was conducted at the delivery room of the Obstetric Hospital, Ain-Shams University, Cairo, Egypt from the period between June 2007 to Feb. 2008. It included 50 appropriate for gestational age Egyptian newborns (25 full-term and 25 preterm newborns). Full-term newborns (37 to 41 weeks gestation) were 11 males and 14 females and their birth weights ranged from 2500 to 4000 g. The preterm infants (30 to 35 weeks gestation) were 17 males and 8 females and their birth weights ranged from 1200 to 2450 g. Gestational age was estimated from the last menstrual period and supported by fetal ultrasound measurements and clinical examination of the neonate according to the Ballard et al. (1991). Out of the total newborns included in the study, 22 (44%) were born via normal vaginal delivery [NVD] (12 full-term and 10 preterm) and 28 (56%) were born via caesarian section [CS] (13 full-term and 15 preterm).

All newborns were healthy. We excluded neonates with major or lethal congenital malformations, prenatal infection, small and large for gestational age and those whose mothers had presentational and gestational diabetes, or preclampsia, or receiving hormonal therapy. Parental consent was obtained from the parents of the studied newborns.

All newborns in the study were subjected to thorough clinical examination with APGAR score at 1 and 5 min together with anthropometric measurements including birth weight using an electronic scale, birth length measured on a wooden measuring board, head and abdominal circumferences using non-stretchable measuring tapes and skin fold thickness (triceps, biceps and subscapular) using Harpenden caliper. Body Mass Index (BMI) was calculated as kg m⁻² squared as the ratio of body weight (kg) and squared height (m²):

\[
\text{BMI} = \frac{\text{Body weight (kg)}}{\text{Height}^2 \text{ (m)}}
\]

**Blood sampling:** Cord blood samples were withdrawn at the time of delivery and before milk feeding. Serum and plasma were separated after centrifugation and stored at -70°C until assay.

**Laboratory investigations:** Serum ghrelin level was determined by DRG® Ghrelin (human) ELISA KIT which is a solid phase Enzyme-Linked Immunosorbent Assay (ELISA) based on the sandwich principle (EIA-3756). DRG International Inc. USA (Porstmann and Kiessing, 1992). Serum leptin level was determined by DRG Leptin (human) ELISA KIT (EIA-2395). DRG International Inc. USA (Considine et al., 1996). Serum insulin level was determined by DRG insulin ELISA Kit (EIA-2935, DRG Instruments GmbH. Germany) (Judzewitsch et al., 1982). Glucose was determined with the glucose hexokinase enzymatic method (Hitachi 917 analyzer, Roche, Indianapolis, IN).

**Statistical analysis:** Statistical Package for Social Sciences (SPSS) program version 11 was used for analysis of data. Data were expressed as Means±SD and percentage. Comparison of means between two different groups was performed using the non-paired student t-test. Correlations were performed using the Pearson bivariate correlation. To verify the influence of different variables on cord ghrelin level, we used multiple regression analysis to determine the effect of independent variables on ghrelin. The p-value was considered significant if <0.05.

**RESULTS**

The study included 25 full-term (11 males and 14 females) and 25 preterm newborns (17 males and 8 females).

Descriptive data of the studied neonates is shown in Table 1. As expected, all anthropometric measurements

| Table 1: Descriptive data of full-term and preterm newborns |
|-------------|-----------------|-----------------|----------|
| Items       | Full-term        | Preterm         | p-value  |
| Gender      | 11/14 (44/32)    | 17/8 (68/32)    | 0.07     |
| Mode of delivery: NVD/CS | 12/13 (48/52)    | 10/15 (40/60)   | 0.38     |
| Gestational age (week) | 38.90±1.10      | 32.84±2.17      | 0.0001*  |
| Weight (g)  | 3.40±3.6        | 2.06±3.83       | 0.0001*  |
| Length (cm) | 49.20±2.33      | 43.56±2.65      | 0.0001*  |
| BMI (kg m⁻²)| 6.79±0.59       | 4.57±0.79       | 0.0001*  |
| Triceps skin fold (mm) | 6.94±1.69      | 5.38±1.24       | 0.001*   |
| Biceps skin fold (mm) | 6.52±2.16       | 5.30±1.29       | 0.019*   |
| Subscapular skin fold (mm) | 6.12±1.69      | 4.32±1.35       | 0.0001*  |
| Abdominal circumference (cm) | 31.48±2.73     | 26.08±2.10      | 0.0001*  |
| Head circumference (cm) | 34.24±1.72     | 30.56±3.22      | 0.0001*  |
| Ghrelin (ng mL⁻¹) | 3.84±2.64      | 3.96±6.26       | 0.86     |
| Leptin (ng mL⁻¹) | 16.59±6.19     | 2.39±1.73       | 0.0001*  |
| Insulin (μU mL⁻¹) | 5.72±3.11      | 7.08±3.91       | 0.18     |
| Glucose (mg dl⁻¹) | 49.00±6.58     | 50.16±6.62      | 0.53     |

Data were expressed as Mean ± SD and except numbers between parentheses. *p-value is significant if <0.05. NVD: Normal vaginal delivery, CS: Caesarian section, BMI: Body mass index.
Table 2: Correlations between ghrelin and other items

<table>
<thead>
<tr>
<th>Items</th>
<th>Total newborns (N = 50)</th>
<th></th>
<th>Full-term (N = 25)</th>
<th></th>
<th>Preterm (N = 25)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>p-value</td>
<td>r</td>
<td>p-value</td>
<td>r</td>
<td>p-value</td>
</tr>
<tr>
<td>Gestational age (week)</td>
<td>0.078</td>
<td>0.288</td>
<td>0.09</td>
<td>0.296</td>
<td>0.036</td>
<td>0.09</td>
</tr>
<tr>
<td>Weight (g)</td>
<td>0.149</td>
<td>0.280</td>
<td>0.161</td>
<td>0.401</td>
<td>0.017</td>
<td>0.09</td>
</tr>
<tr>
<td>Length (cm)</td>
<td>0.098</td>
<td>0.249</td>
<td>0.209</td>
<td>0.041</td>
<td>0.127</td>
<td>0.04</td>
</tr>
<tr>
<td>BMI (kg m⁻²)</td>
<td>0.166</td>
<td>0.134</td>
<td>0.141</td>
<td>0.305</td>
<td>0.340</td>
<td>0.340</td>
</tr>
<tr>
<td>Triceps skin fold (mm)</td>
<td>0.239</td>
<td>0.091</td>
<td>0.047</td>
<td>0.488</td>
<td>0.027</td>
<td>0.771</td>
</tr>
<tr>
<td>Biceps skin fold (mm)</td>
<td>-0.218</td>
<td>0.507</td>
<td>-0.013</td>
<td>0.315</td>
<td>0.125</td>
<td>0.065</td>
</tr>
<tr>
<td>Subscapular skin fold (mm)</td>
<td>0.409</td>
<td>0.198</td>
<td>0.276</td>
<td>0.375</td>
<td>0.065</td>
<td>0.771</td>
</tr>
<tr>
<td>Abdominal circumference (cm)</td>
<td>0.100</td>
<td>0.011</td>
<td>0.206</td>
<td>0.428</td>
<td>0.003</td>
<td>0.785</td>
</tr>
<tr>
<td>Head circumference (cm)</td>
<td>0.142</td>
<td>0.242</td>
<td>0.052</td>
<td>0.375</td>
<td>0.065</td>
<td>0.771</td>
</tr>
<tr>
<td>Leptin (ng mL⁻¹)</td>
<td>0.258</td>
<td>0.063</td>
<td>0.114</td>
<td>0.350</td>
<td>0.086</td>
<td>0.350</td>
</tr>
<tr>
<td>Insulin (mIU mL⁻¹)</td>
<td>0.409</td>
<td>0.019</td>
<td>0.276</td>
<td>0.375</td>
<td>0.065</td>
<td>0.771</td>
</tr>
<tr>
<td>Glucose (mg dl⁻¹)</td>
<td>-0.409</td>
<td>0.063</td>
<td>-0.111</td>
<td>0.036</td>
<td>0.864</td>
<td>0.036</td>
</tr>
</tbody>
</table>

*p-value is significant if ≤0.05. BMI: Body mass index.

Table 3: Correlations between leptin and other items

<table>
<thead>
<tr>
<th>Items</th>
<th>Total newborns (N = 50)</th>
<th></th>
<th>Full-term (N = 25)</th>
<th></th>
<th>Preterm (N = 25)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>p-value</td>
<td>r</td>
<td>p-value</td>
<td>r</td>
<td>p-value</td>
</tr>
<tr>
<td>Gestational age (week)</td>
<td>0.538</td>
<td>0.0001*</td>
<td>-0.264</td>
<td>0.161</td>
<td>0.442</td>
<td>0.0001*</td>
</tr>
<tr>
<td>Weight (g)</td>
<td>0.632</td>
<td>0.0001*</td>
<td>0.155</td>
<td>0.155</td>
<td>0.988</td>
<td>0.038*</td>
</tr>
<tr>
<td>Length (cm)</td>
<td>0.560</td>
<td>0.0001*</td>
<td>0.107</td>
<td>0.104</td>
<td>0.999</td>
<td>0.0004*</td>
</tr>
<tr>
<td>BMI (kg m⁻²)</td>
<td>0.601</td>
<td>0.0001*</td>
<td>0.114</td>
<td>0.350</td>
<td>0.086</td>
<td>0.350</td>
</tr>
<tr>
<td>Triceps skin fold (mm)</td>
<td>0.276</td>
<td>0.053</td>
<td>-0.101</td>
<td>0.328</td>
<td>0.110</td>
<td>0.328</td>
</tr>
<tr>
<td>Biceps skin fold (mm)</td>
<td>0.352</td>
<td>0.012*</td>
<td>0.211</td>
<td>0.142</td>
<td>0.490</td>
<td>0.142</td>
</tr>
<tr>
<td>Subscapular skin fold (mm)</td>
<td>0.454</td>
<td>0.001*</td>
<td>0.184</td>
<td>0.424</td>
<td>0.055*</td>
<td>0.424</td>
</tr>
<tr>
<td>Abdominal circumference (cm)</td>
<td>0.649</td>
<td>0.0001*</td>
<td>0.343</td>
<td>0.525</td>
<td>0.007*</td>
<td>0.525</td>
</tr>
<tr>
<td>Head circumference (cm)</td>
<td>0.431</td>
<td>0.002*</td>
<td>0.067</td>
<td>0.329</td>
<td>0.108</td>
<td>0.329</td>
</tr>
<tr>
<td>Ghrelin (ng mL⁻¹)</td>
<td>-0.025</td>
<td>0.863</td>
<td>-0.052</td>
<td>0.277</td>
<td>0.180</td>
<td>0.277</td>
</tr>
<tr>
<td>Insulin (mIU mL⁻¹)</td>
<td>0.030</td>
<td>0.839</td>
<td>0.030</td>
<td>0.188</td>
<td>0.369</td>
<td>0.188</td>
</tr>
<tr>
<td>Glucose (mg dl⁻¹)</td>
<td>-0.114</td>
<td>0.430</td>
<td>-0.111</td>
<td>0.036</td>
<td>0.864</td>
<td>0.036</td>
</tr>
</tbody>
</table>

*p-value is significant if ≤0.05. BMI: Body mass index.

An overall significant correlation was found between leptin, gestational age, weight, length, BMI, biceps and subscapular skin fold thickness as well as abdominal and head circumferences. Also, leptin correlated with weight, length, subscapular skin fold thickness and abdominal circumferences in preterm neonates (r = 0.418, p = 0.038; r = 0.555, p = 0.004; r = 0.424, p = 0.035, r = 0.525, p = 0.007, respectively) (Table 3).

Insulin correlated with subscapular skin fold thickness in term neonates (r = 0.405, p = 0.045).

**DISCUSSION**

Ghrelin may play a possible role during intrauterine life, especially in determining adaptations of the fetus to an adverse intrauterine environment (Korbonits et al., 2004).

In the current study, umbilical cord ghrelin and leptin were detectable in the whole population of the study as early as 30 weeks gestation. This is in agreement with Ng et al. (2005) who detected ghrelin as early as 23 weeks gestation indicating that ghrelin mechanism is present in intrauterine life and even in premature age. The existence of ghrelin in cord blood is also compatible with previous observations (Kitamura et al., 2003; Chanoine et al., 2002).
Cortelazzi et al. (2003) demonstrated the presence of ghrelin in fetal circulation from the 20th week of gestation to term and stated that these ghrelin levels are produced by the fetus. Furthermore, previous studies of the human fetus found that ghrelin-immunoreactive cells were well displayed in the stomach, duodenum, pancreas and lung from the 10th week of gestation (Rindi et al., 2002; Volante et al., 2002).

Also, it is possible that some of the ghrelin in the fetal circulation might originate from the placenta, like leptin and regulate feto-maternal energy transport locally (Kitanuma et al., 2003) since ghrelin mRNA is expressed in the human placenta (Gualillo et al., 2001). It was found that ghrelin concentrations in cord blood were significantly higher in the vein than in the artery and suggesting the placenta as an important source of fetal ghrelin (Kitanuma et al., 2003). Yokota et al. (2005) further demonstrated the existence of octanoylated ghrelin in fetal and neonatal circulation.

Our study revealed no significant difference between full-term and preterm neonates as regards umbilical cord ghrelin level, which is in agreement with several investigators (Soriano-Guillén et al., 2004; Ng et al., 2005). Other studies demonstrated that umbilical cord ghrelin was higher in term than preterm infants (Bellone et al., 2004) or higher in preterm infants (Siahnanidou et al., 2005) but their study was differ from our study is that their study done postrnatally after milk feeding and the investigators attributed this to increased synthesis/secretion and/or to decreased clearance of these peptides.

We found no overall correlation between ghrelin and gestational age or anthropometric measurements which is in accord with other researchers (Ng et al., 2005).

Also, we did not find any correlation between ghrelin and gestational age in both full-term and preterm subgroups which is in agreement with other investigators (Yokota et al., 2005; Bellone et al., 2003, 2006), who reported that ghrelin secretion did not appear to undergo gestational age-related variations since, they found that ghrelin concentrations were relatively constant at birth supporting the observation that ghrelin secretion is relatively constant with age.

Another study revealed an inverse relationship between ghrelin and gestational age but this study was done on SGA, so ghrelin level might be affected by a confounding pathological factor (Farquhar et al., 2003).

The current study revealed no correlation between ghrelin concentration and anthropometric indices in full-term group, which is similar to previous studies (James et al., 2004; Pirazzoli et al., 2005). On the contrary, other investigators found that cord blood ghrelin was inversely related with birth weight and birth length and BMI, suggesting that ghrelin concentration might be mainly regulated in a fetal growth-related manner in utero (Ng et al., 2005; Onal et al., 2004) and that the metabolic hormonal system is probably operational in fetal life. It was stated that this phenomenon could be beneficial to term newborns by stimulating their appetite and maintaining an adequate blood sugar level at the most critical period when nutrients from mothers are abruptly terminated after birth (Ng et al., 2005).

As regards the preterm group we found a significant correlation between cord ghrelin and birth weight, BMI and abdominal circumference. Other investigators found that none of the anthropometric measurements they studied correlated with serum ghrelin concentrations in preterm infants (Soriano-Guillén et al., 2004; Ng et al., 2005).

The controversy concerning the relationship between ghrelin and anthropometric indices at birth may be attributed to different population categories, nutritional status, different Kits used in the assay or different techniques used.

We didn’t find any correlation between ghrelin and leptin, insulin or glucose neither in the whole newborn nor in both term and preterm subgroups which is similar to other studies that failed also to find such correlation (Ng et al., 2005; James et al., 2004; Lányi et al., 2008). Our findings agree with the opinion of many authors who believe that ghrelin and leptin function are unlikely to be linked by a functional relationship; despite the fact that both play relevant actions in the control of appetite and energy expenditure (Van der Lely et al., 2004); these hormones do not seem linked by direct functional feedback (Chan et al., 2004).

It was reported that the lack of any direct relationship between ghrelin and anthropometric or biochemical parameters in adequate for gestational age newborns does not support the hypothesis that ghrelin has major role in fetal growth (Bellone et al., 2004).

Other researchers found a strong negative association between ghrelin and insulin levels. It appears that insulin may suppress circulating ghrelin levels (Flanagan et al., 2003; Purnell et al., 2003).

The current study, showed that there was no sex related differences regarding anthropometric measurements and serum levels of ghrelin, leptin, insulin or glucose, which is consistent with other studies which reported that circulating ghrelin levels in cord blood of newborns are independent of gender (Ng et al., 2005) (Lányi et al., 2008). Alternatively, other investigators found that female infants had significantly higher ghrelin
and leptin levels than male infants and suggested that sexual dimorphism for ghrelin might exist in the perinatal period. However, they found no significant difference in serum insulin between the two sexes (Ng et al., 2004). Also, previous studies revealed a sex difference with higher serum leptin in female than in male infants (Ng et al., 2000; Ong et al., 1999). Underlying causes might be the differential amount of fat tissue by gender, the role of the variable sex steroid milieu of the newborn and the heavier placental weight associated with female gender (Petridou et al., 2005).

Also, we found that mode of delivery whether NVD or CS didn’t make any difference regarding ghrelin, leptin and insulin levels which is in agreement with other investigators (Láinyi et al., 2008).

Leptin is probably one of the most crucial hormones responsible for weight and fat regulation in uterus. It regulates intrauterine and early extrauterine life growth and development, as well as the adaptation to extrauterine life (Ng et al., 2004; Alexe et al., 2006).

The current study showed that umbilical cord leptin was significantly lower in preterm newborns than full-terms; which reflect a lower fat mass content compared to full-term newborns. This result is contributed to the fact that the adipose tissue is important source for leptin which increases with gestation in parallel with increase in the adipose mass (Ng et al., 2005; Alexe et al., 2006; Valiniente et al., 2007).

The results of previous studies have revealed that the capacity of fetal adipocytes to synthesize leptin is relatively limited until late in gestation, while the placenta synthesizes little if any leptin (Amico et al., 1998). Also, it has been stated that this placental role decreases during late pregnancy in parallel with an upregulation of expression of the shorter isoforms of the leptin receptor in the placenta (Smith and Waddell, 2003).

We found an overall significant correlation between leptin and gestational age, birth weight, length, BMI, biceps and subscapular skin fold thickness as well as abdominal and head circumferences which was similar to other studies (Ng et al., 2004; Stoll-Beeker et al., 2003; Chiesa et al., 2008). Also, it correlated with weight, length, subscapular skin fold thickness and abdominal circumference in preterm neonates suggesting a role in fat regulation in utero. Other investigators found that leptin was correlated with birth weight in full-term newborns (James et al., 2004). Furthermore, previous studies showed a correlation between leptin concentrations and weight in both preterm and full-term infants (Yildiz et al., 2002); suggesting a pivotal role of fetal leptin in regulating fetal growth and development.

The present study, showed no overall significant correlation between leptin and ghrelin, insulin or glucose and neither in both subgroups. Conversely, other investigators found that leptin was negatively associated with plasma glucose but their study category was different (Ng et al., 2004). Also, a previous study of the longitudinal profile of leptin and metabolic hormones in preterm infants revealed that serum leptin was significantly associated with serum insulin and insulin: glucose ratio supporting the hypothesis that an adipocinsinal axis exists and is likely to be functional before 34 weeks of gestation (Ng et al., 2001).

As we see, leptin undergo gestational age related variations unlike ghrelin which is in accordance with several investigators (Ng et al., 2005; Stoll-Beeker et al., 2003). Also, unlike ghrelin, leptin showed association with common anthropometric parameters which is consistent with other studies (Ng et al., 2004; Chiesa et al., 2008).

It has to be taken into consideration the fact that we (as well as other investigators) assessed only total ghrelin and not the octanoylated ghrelin; so, the physiological role of ghrelin in newborns remains to be clarified using kits to assess octanoylated ghrelin. Also, further studies of ghrelin levels in newborns with pathological states may provide valuable information about its role in neonatal period.

CONCLUSIONS

Umbilical cord ghrelin was detectable in all newborns included in the study as early as 30 weeks gestation and was relatively invariable at birth. It might undertake its active physiological role in regulation of growth and metabolism from a relatively early stage of gestation and continues throughout the rest of the pregnancy. The lack of clinically significant correlations between ghrelin and gestational age, suggest that ghrelin secretion might not undergo gestational age related variations.

Lower leptin levels in preterm compared to full-term groups indicates increased adipose mass and placental maturation with increased gestational age.

REFERENCES


