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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-residue 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 been also detected in cord blood (Chanoine *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; Broglio *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-residue 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 Jeanrenand, 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 (m^2):

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

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-3706). 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 Mean±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 (N = 25)	Preterm (N = 25)	p-value
Gender			
Male:Female (N and %)	11/14 (44/56)	17/8 (68/32)	0.07
Mode of delivery NVD/CS	12/13 (48/52)	10/15(40/60)	0.38
Gestational age (week)	38.96±1.10	32.84±2.17	0.0001*
Weight (g)	3340±346	2006±433	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	3.966±2.36	0.86
Leptin (mg mL^{-1})	16.596±11.69	2.39±1.73	0.0001*
Insulin (μiu mL^{-1})	5.72±3.11	7.08±3.91	0.18
Glucose (mg dL^{-1})	49.00±6.98	50.16±6.62	0.55

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

	Total newborns (N = 50)		Full-term (N = 25)		Preterm (N = 25)	
Items	r	p-value	r	p-value	r	p-value
Gestational age (week)	0.078	0.588	0.009	0.965	0.346	0.09
Weight (g)	0.149	0.302	0.289	0.161	0.401	0.047*
Length (cm)	0.098	0.498	0.045	0.829	0.314	0.127
BMI (kg m^{-2})	0.166	0.249	0.308	0.134	0.413	0.04*
Triceps skin fold (mm)	0.221	0.123	0.345	0.091	0.146	0.485
Biceps skin fold (mm)	-0.118	0.415	-0.013	0.950	-0.313	0.127
Subscapular skin fold (mm)	0.254	0.076	0.392	0.053	0.199	0.340
Abdominal circumference (cm)	0.100	0.489	0.011	0.960	0.428	0.033*
Head circumference (cm)	0.142	0.324	0.026	0.901	0.315	0.125
Leptin (mg mL^{-1})	-0.025	0.863	-0.052	0.806	0.375	0.065
Insulin (μiu mL^{-1})	0.099	0.494	0.276	0.182	-0.061	0.771
Glucose (mg dL^{-1})	-0.086	0.551	-0.218	0.295	0.063	0.765

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

Table 3: Correlations between leptin and other items

	Total newborns (N = 50)		Full-term (N = 25)		Preterm (N = 25)	
Items	r	p-value	r	p-value	r	p-value
Gestational age (week)	0.538	0.0001*	-0.264	0.201	0.161	0.442
Weight (g)	0.622	0.0001*	0.155	0.458	0.418	0.038*
Length (cm)	0.560	0.0001*	0.108	0.609	0.555	0.004*
BMI (kg m^{-2})	0.601	0.0001*	0.114	0.587	0.350	0.086
Triceps skin fold (mm)	0.276	0.053	-0.101	0.630	0.328	0.110
Biceps skin fold (mm)	0.352	0.012*	0.211	0.311	0.142	0.499
Subscapular skin fold (mm)	0.454	0.001*	0.184	0.379	0.424	0.035*
Abdominal circumference (cm)	0.649	0.0001*	0.343	0.093	0.525	0.007*
Head circumference (cm)	0.431	0.002*	0.067	0.752	0.329	0.108
Grhelin (ng mL^{-1})	-0.025	0.863	-0.052	0.806	0.277	0.180
Insulin (μiu mL^{-1})	0.030	0.839	0.307	0.136	0.188	0.369
Glucose (mg dL^{-1})	-0.114	0.430	-0.111	0. 596	0.036	0.864

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

(weight, length, BMI, head and abdominal circumference as well as skin fold thickness) were significantly, reduced in preterm neonates than the full-term group ($p<0.05$). There was no significant difference between full-term and preterm groups as regards umbilical cord ghrelin, insulin and glucose. On the other hand, umbilical cord leptin was significantly reduced in preterm neonates compared to full-terms ($p=0.0001$).

There was no significant sex related differences regarding anthropometric measurements and serum levels of ghrelin, leptin, insulin or glucose ($p>0.05$).

Mode of delivery whether NVD or CS did not make any significant difference concerning anthropometric measurements, ghrelin, leptin, insulin or glucose levels ($p>0.05$).

Table 2 demonstrates that there was no overall correlation between cord ghrelin and gestational age, anthropometric measurements and leptin, insulin or glucose. The same was found in full-term newborns. But, preterm group demonstrated significant correlations between ghrelin and weight, BMI and abdominal circumference ($r = 0.401$, $p = 0.047$; $r = 0.413$, $p = 0.04$; $r = 0.428$, $p = 0.033$, respectively); with abdominal circumference being the most determinant variable for ghrelin level by regression analysis ($R^2 = 0.183$, $\beta = 0.428$, $p = 0.033$).

An overall significant correlation was found between leptin and 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 (Kitamura *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 (Kitamura *et al*., 2003). Yokota *et al*. (2005) further demonstrated the existence of octanoylted 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 (Siahanidou *et al*., 2005) but their study was differ from our study is that their study done postnatally 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 correlated 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 newborns 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 milieus 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 (Lányi *et al*., 2008).

Leptin is probably one of the most crucial hormones responsible for weight and fat regulation in utero. 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; Valûniene *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 increases 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-Becker *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 adipoinsular 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-Becker *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.

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