Biomedical & Pharmacology Journal, September 2019. Vol. **12**(3), p. 1147-1154

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

Enas R. Abdelhamid[1], Alyaa H. Kamhawy[1]*, Hanaa H. Ahmed[2], Mones M. Abu Shady[1], Ahmed Fathy[1] and Reham F. Fahmy[1]

[1]Department of Childhealth, Medical Division, National Research Center (NRC), Cairo, Egypt, 33rd El Bohouth st, Former El Tahrir st, Dokki, Giza, POB:12311, Egypt.
[2]Department 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 anrthropometric 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[1,2] . When born either SGA or LGA may strength the risks for metabolic syndrome, obesity, T2DM, and CVD in adult life[3,4] . It has been reported that about 10 % of newborn babies in the US have birth weight aberrations either SGA or LGA[5] The onset of macrosomia and LGA has been reported to be enhanced over decennium in numerous nations[6,7]. LGA is correlated with increased risks of neonatal morbidity, neonatal and maternal harm as well as Caesarean section delivery[8]. The factors affecting birth weight include genetics, gestational diabetes, duration of gestation, and ethnic factors[9]. Preceding reports mentioned that cord blood ghrelin levels have a negative correlation with body weight in SGA[10,11] . 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 fetalgrowth and participatein birth weight establishment[12,13]

Firstly ,Ghrelin was recognized as the endogenousligand for growth hormone secretagogue receptor(GHS-R1) [14] Ghrelin (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 itsinfluence on GH release , and thus it was suggested to be influential in growth[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[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[17].

Several other studies have also been related to ghrelin and its effect in growth[18,19].Apart from its action on GH realease, ghrelin has been found to increase appetite and initiate adiposity[20]. Infant with SGAnewborn infants experienced an inhibited rate of development during the intrauterine interval , which might be attributedto fetal, maternal or environmental factors. These infants have a decreased concentrations of GH after delivery as a result of nutritionalshortage before birth[16].

In spite of most of theneonates 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 orexigenicaction 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[23,10].

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 umblicalcord 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 fulltermbabiesfrom normal delivery or cesarean section, without any birth complications including perinatal asphyxia, or acute fetalsuffering signs were included in this research. Exclusion criteria included womenpresenting with diabetes,preeclampsia,

antiphospholipidsyndrome, connectivetissue diseases, chronic infection, alcoholism, orsmoking during the pregnancy .

Newborns were assigned inaccordanceto their birth weight in SGA, (lowest 10th percentile),AGA(between 10th and 90th percentile), and LGA(higher 90th percentile).We collected clinical and anthropometrical informatiosfrom mothers and newborns by direct interview and clinical sheets . Maternal blood samples were withdrawn during laborafter beingfasting 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 measuredby 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 1. AnthropometricPatterns of SGA, AGA, and LGA newborns

variable	SGA	AGA mean± SD	LGA	F-value	P-value
MotherAge	26.13±5.80	25.76±4.89	26.38±5.32	0.064	0.938
Mother weight	71.929±16.905	76.620±15.847	84.977±9.343	3.104	0.052
Mother height	157.25±5.02	158.16±6.95	160.54±7.33	1.139	0.327
Neonatal weight	1.9694±0.3563	3.0532±0.2167	4.1154±0.4518	138.713	0.000*
Neonatal length	43.21±3.12	43.21±3.12	51.15±2.15	53.591	0.000*
HC	31.71±1.71	34.84±1.07	36.15±1.34	51.724	0.000*
MAC	8.604±1.103	10.360±.941	12.000±1.000	49.110	0.000*
BMI	28.9927±6.2190	30.6347±5.8594	33.1148±4.2766	2.195	0.120

HC:head circumference
MAC:mean arm circumference
BMI:body mass index
$P < 0.05$ is significant

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

Variable	SGA mean± SD	AGA	LGA	F-value	P-value
Gestational age	37.83±1.52	37.68±0.95	37.00±1.73	1.637	0.203
Apgar score at the first minnute	4.64±1.15	6.24±1.51	5.00±1.49	6.115	0.005*
Apgar score at the 5th minute	6.79±1.19	8.57±1.25	8.10±1.29	8.884	0.001*
Maternal des-acyl ghrelin (pg/ml)	731.30±235.47	627.50±231.25	684.62±149.70	0.744	0.482
Cord blood des-acyl ghrelin (pg/ml)	662.61	500.00	524.62	4.854	0.013*
$P < 0.05$ is significant	183.93	75.59	140.04		

Table (2) shows a significant difference between SGA,AGA, and LGA as regardingapgar 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 anrthropometricmeasures(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

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 withthat of Dinget 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

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

	Unstandardized Coefficients		Standardized Coefficients	t	Sig.
	B	Std. Error	Beta		
(Constant)	5713.755	4170.705		1.370	.188
Mother weight	32.376	24.185	3.346	1.339	.197
Mother height	-23.389	24.742	-.829	-.945	.357
Neonatal weight	-210.470	67.153	-1.224	-3.134	.006*
Neonatal length	41.137	22.886	.905	1.797	.089
HC	5.020	28.990	.065	.173	.864
MAC	-49.806	36.843	-.478	-1.352	.193
Apgar score at the first minnute	-92.270	33.879	-.817	-2.723	.014*
Apgar score at the 5th minute	35.623	35.570	.307	1.001	.330
Gestational age	-47.894	18.198	-.392	-2.632	.017*
Maternal des-acyl ghrelin (pg/ml)	-4.992	.111	-.069	-.448	.660

Table 4. Correlation between cord ghrelin and neonatal variables

		Neonatal weight	Neonatal length	HC	MAC	Apgar score at the first minnute	Apgar score at the 5th minute	Ghrelin in mother (pg/ml)
Cord blood	r	-.479(**)	-.376(*)	-.480(**)	-.412(**)	-.359(*)	-.412(*)	-.095
des-acyl ghrelin	p	.001	.012	.001	.005	.047	.021	.542

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

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[11, 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 thisstatus 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 Dinget 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 mid-gestation 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 waistof the neonate[38] 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 Dinget al. who stated that cord blood ghrelin levels and body length, head circumference and BMI werenegatively 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]. thuselevating the risk of metabolic disorders in future life. A drop in DAG and total ghrelin have been recorded both in metabolically abnormal children andhealthy 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 prepuberalnon-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 withDinget al. who mentioned that the body weight is the essential actor correlated with cord ghrelin levels[31] .

In our study ,the level of des-acyl ghrelin doesn't differ between both gender ,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[10,45]

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 5min[46].

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" 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 andnutritional 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.

AKNOWLEDGEMENT:

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

1. Das U.G ,Sysyn G.D .Abnormal fetal growth: intrauterinegrowth retardation, small for gestational age, large for gestational age. Pediatr. Clin. North Am.2004 ; 51(3), 639–654.
2. Carrascosa A, Yeste D, Copil A, Audi L , Gusinye M , Vicens-Calvet E , Clemente M . Fetal growth regulation and intrauterine growth retardation. J. Pediatr. Endocrinol. Metab. 2004 ;17(Suppl 3), 435–443 .
3. de Boo HA , Harding JE .The developmental origins of adultdisease (Barker) hypothesis. Aust. N. Z. J. Obstet. Gynaecol. 2006; 46(1), 4–14 .
4. Xita N., Tsatsoulis A. Fetal origins of the metabolic syndrome. Ann. N. Y. Acad. Sci.2010 ;1205, 148–155 .
5. Donahue SM, Kleinman KP, Gillman MW, Oken E .Trendsin birth weight and gestational length among singleton term births in the United States: 1990–2005. Obstet. Gynecol.2010; 115(2 Pt 1), 357–364
6. Arbuckle TE, Sherman GJ .An analysis of birth weight by gestational age in Canada. *CMAJ*. 1989 ;140: 157–160, 165.
7. Lu Y, Zhang J, Lu X, Xi W, Li Z. Secular trends of macrosomia in southeast China, 1994–2005. BMCPublic Health 2011 ;11: 818.
8. Spellacy WN, Miller S, Winegar A, Peterson PQ .Macrosomia– maternal characteristics and infant complications. *ObstetGynecol* . 1985. ; 66: 158–161.
9. Okun N, Verma A, Mitchell BF, FlowerdewG.. Relative importance of maternal constitutional factors and glucose intolerance of pregnancy in the development of newborn macrosomia. *J Matern Fetal Med* .1997 ;6: 285–290.
10. Onal EE, Cinaz P, AtalayY .Umbilical cord ghrelin concentrations in small- and appropriate-for-gestational age newborn infants: relationship to anthropometric markers. *J Endocrinol*. 2004; 180:267–271.
11. Farquhar J, Heiman M, Wong AC, Wach R, Chessex P, Chanoine JP. .Elevated umbilical cord ghrelin concentrations in small for gestational age neonates. *J ClinEndocrinolMetab* . 2003.;88(9):4324–7
12. GualilloO, Caminos J, Blanco M, Garcia-Caballero T, Kojima M, Kangawa K, Dieguez C, Casanueva F. Ghrelin, a novelplacental-derived hormone. Endocrinology. 2001; 142(2), 788–794

13. Kitamura S, Yokota I, Hosoda H, Kotani Y, Matsuda J, Naito E, Ito M, Kangawa K, Kuroda Y ..Ghrelin concentration in cord and neonatal blood: relation to fetal growth and energybalance. J. Clin. Endocrinol. Metab.2003; 88(11), 5473–5477.
14. Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. Nature1999;. 402(6762), 656–660.
15. Cunha SR, Mayo KE .Ghrelin and growth hormone (GH) secretagogues potentiate GH-releasing hormone (GHRH)-induced cyclic adenosine 3',5'-monophosphate production in cells expressing transfected GHRH and GH secretagogue receptors. *Endocrinology* 2002 ;143(12):4570–82.
16. VeldhuisJD ,Bowers CY. .Integrating GHS into the Ghrelin System. *Int J Pept.* Vol.2010, Article ID 879503DOI:10.1155/2010/879503
17. Delhanty PJ, Neggers SJ, van der Lely AJ ..Mechanisms inendocrinology: ghrelin: the differences between acyl- and desacylghrelin. Eur. J. Endocrinol. .2012; 167(5), 601–608 .
18. Casanueva FF, Camina JP, Carreira MC, Pazos Y, Varga JL, Schally AV .Growth hormone-releasing hormone as an agonist of the ghrelin receptor GHS-R1a. *ProcNatlAcadSci U S A.*. 2008;105(51):20452–7.
19. Peroni CN, Hayashida CY, Nascimento N, Longuini VC, Toledo RA, Bartolini P, et al,2012. Growth hormone response to growth hormonereleasingpeptide-2 in growth hormone-deficient little mice. *Clinics(Sao Paulo)*2005.;67(3):265–72 .DOI:10.6061/clinics/2012(03)11
20. Makino Y, Hosoda H, Shibata K, Makino I, Kojima M, Kangawa K . Alteration of plasma ghrelin levels associated with the blood pressure in pregnancy. *Hypertension.*2002 ;39(3):781–4.
21. Luque RM, Kineman RD, Park S, Peng XD, Gracia-Navarro F, Castano JP .Homologousandheterologous regulation of pituitary receptors for ghrelin and growth hormone-releasing hormone. *Endocrinology.*2004 ; 145(7):3182–9.
22. Rasmussen KM, Yaktine AL, Guidelines C, Board FN, Board on Children YF, Medicine I .Weight Gain During Pregnancy: Reexamining the Guidelines.Washington (DC): National Academies Press(US); 2009.PMID:20669500
23. Iniguez G, Ong K, Pena V, Avila A, Dunger D, Mericq V, Fasting and post-glucose ghrelin levels in SGA infants: relationships with size and weight gain at one year of age. *J ClinEndocrinolMetab.* 2002;87(12):5830–3.
24. Cummings D, Purnell JQ, Frayo S, Schmidova K, Wisse BE, WeigleDS .A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans. Diabetes. 2001; 50:1714–1719
25. LeSauter J, Hoque N, Weintraub M, Pfaff DW, Silver R. Stomach ghrelin-secreting cells as food-entrainablecircadianclocks. ProcNatlAcadSci;U S A; 106: 13582–13587 , 2009 .
26. Broglio F, Arvat E, Benso A, Gottero C, Muccioli G, Papotti M, van der Lely AJ, Deghenghi R, Ghigo E. Ghrelin, a natural GH secretagogue producedby the stomach, induces hyperglycemia and reduces insulin secretion in humans.J ClinEndocrinolMetab86 . 2001;5083–5086
27. Cummings DE, Clement K, Purnell JQ, Vaisse C, Foster KE, Frayo RS, Schwartz MW, Basdevant A, WeigleDS .Elevated plasma ghrelin levelsin PraderWilli syndrome. Nat Med .2002 ; 8:643–644
28. Razzaghy-AzarM.Total and Acylated Ghrelin Levels in Children and Adolescents with Idiopathic Short Stature and Poor Appetite. *ActaEndocrinologica.* .2015 ;11(3):401–6.
29. Ariyasu H, Takaya K, Tagami T, Ogawa Y, Hosoda K, Akamizu T, et al. Stomach is a major source of circulating ghrelin, and feeding state determines plasma ghrelin-like immunoreactivity levels in humans. *J ClinEndocrinolMetab.* . 2001;86(10):4753–8.
30. Martha I. Gonzalez-Domýnguez , Maria-Luisa Lazo-de-la-Vega-Monroy , Silvio Zaina , Myrna Sabanero , LeonelDaza-Benýtez ,Juan Manuel Malacara ,Gloria Barbosa-Sabanero, .Association of cord blood des-acyl ghrelin with birth weight, and placental GHS-R1 receptor expression in SGA, AGA, and LGA newborns . Endocrine.2016; 53:182–191.DOI:10.1007/s12020-015-0833-1.
31. Ding H, Pan Y, Yu Y and GuP .Cord ghrelin levels are decreased in large-for-gestational-age neonates. clinicalobesity.2012; 50–56 .DOI:10.1111/j.1758-8111.2012.00034.x.
32. Chiesa C, Osborn J.F, Haass C, Natale F, SpinelliM ,Scapillati E, Spinelli A , Pacifico L .Ghrelin, leptin, IGF-1, IGFBP-3, and insulin concentrations at birth: is there a relationship with fetal growth and neonatal anthropometry? Clin. Chem. 2008;54(3), 550–558 .
33. Jain V, SinghalA . Catch up growth in low birth weight infants: striking a healthy balance. Rev. Endocr. Metab. Disord. . 2012; 13(2), 141–147.
34. Fidanci K, Meral C, Suleymanoglu S, Pirgon O, Karademir F, Aydinoz S, Ozkaya H, Gultepe M, Gocmen I. Ghrelin levels and postnatal growth in healthy infants 0–3 months of age. J. Clin. Res.

Pediatr. Endocrinol..2010 ; 2(1), 34–38

35. Gohlke B.C, Huber A, Hecher K, Fimmers R, Bartmann P, Roth CL .. Fetal insulin-like growth factor (IGF)-I, IGF-II, and ghrelin in association with birth weight and postnatal growthin monozygotic twins with discordant growth. J. Clin. Endocrinol. Metab . 2005; 90(4), 2270–2274.
36. Mendez-Ramirez F, Barbosa-Sabanero G, Romero-Gutierrez G, MalacaraJM . Ghrelin in small-for-gestational age (SGA) newborn babies: a cross-sectional study. Clin. Endocrinol. (Oxf.) 70(1), 41–46 .2009 .
37. Fuglsang J, Skjaerbaek C, Espelund U, Frystyk J, Fisker S, Flyvbjerg A, Ovesen P .Ghrelin and its relationship to growth hormones during normal pregnancy. Clin. Endocrinol. (Oxf.) 62(5), 554–559 ; 2005.
38. Valsamakis G., Papatheodorou D.C., Naoum A., Margeli A., Papassotiriou I., Kapantais E., Creatsas G., Kumar S., MastorakosG.. Neonatal birth waist is positively predicted by secondtrimester maternal active ghrelin, a pro-appetite hormone, and negatively associated with third trimester maternal leptin, a prosatiety hormone. Early Human Dev.2014; 90(9), 487–492.ckwell Publishing, Ltd.
39. Martos-Moreno GA, Barrios V, Barrios V, Saenz dePipaon M , Pozo J, Dorronsoro I , Martinez-Biarge M , Quero J .Influence of prematurity and growth restriction on the adipokineprofile, IGF1, and ghrelin levels in cord blood: relationship with glucose metabolism. Eur. J. Endocrinol. . 2009; 161(3), 381–389 .
40. Bellone S, Rapa A, Vivenza D , Vercellotti A, Petri A, Radetti G, Bellone J, Broglio F, Ghigo E, Bona G. Circulating ghrelin levels in the newborn are positively associated with gestational age. Clin. Endocrinol. (Oxf.) 60(5), 613–617; 2004.
41. Bocca-Tjeertes I.F, Kerstjens J.M, Reijneveld S.A, Veldman K,Bos A.F, de Winter A.F. Growth patterns of large for gestational age children up to age 4 years. Pediatrics 133(3), e643–649; 2014.
42. Mingrone G, Manco M, Mora ME, Guidone C, Iaconelli A,Gniuli D, Leccesi L, Chiellini C, Ghirlanda G .Influence of maternal obesity on insulin sensitivity and secretion in offspring. Diabetes Care . 2008; 31(9), 1872–1876
43. Pacifico L, Poggiogalle E, Costantino F, Anania C, Ferraro F, Chiarelli F, Chiesa C ..Acylated and nonacylated ghrelin levels and their associations with insulin resistance in obese and normal weight children with metabolic syndrome. Eur. J. Endocrinol. 2009 ; 161(6), 861–870
44. Darendeliler F, Poyrazoglu S, Bas F, Sancakli O , Gokcay G. .Ghrelin levels are decreased in non-obese prepubertal children born large for gestational age. Eur. J. Endocrinol. .2009; 160(6), 951–956 .
45. BelloneS ,Prodam F, Savastio S, Avanzo D, Pagani A, Trovato L, Walker G E, Genoni G and Bona G. Acylated/unacylated ghrelin ratio in cord blood: correlation with anthropometric and metabolic parameters and pediatriclifespan comparison. Journal of Endocrinology. 2012 ;166 :115–120.DOI:10.1530/EJE-11-0346.
46. Pak C. Ng, Cheuk H. Lee, Christopher W. K. Lam, Iris H. S. Chan, Eric Wong and Tai F. Fok. Ghrelin in preterm and term newborns:relation to anthropometry, leptin and insulin Clinical Endocrinology 63 , 217–222.