Maternal serum and cord blood leptin concentrations in the third trimester of normal pregnancy

Małgorzata Stefaniak[1], Ewa Dmoch-Gajzlerska[1], Barbara Mazurkiewicz[1], Wanda Gajzlerska-Majewska[2]

[1]Department of Obstetrics and Gynecology Didactics, Medical University of Warsaw, Poland

[2] Department of Obstetrics and Gynecology, Medical University of Warsaw, Poland

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Corresponding address:
Małgorzata Stefaniak
Medical University of Warsaw, Faculty of Health Sciences, Department of Obstetrics and Gynecology Didactics
Zwirki i Wigury St. 81
02-091 Warsaw
e-mail: malgorzata.stefaniak@wum.edu.pl
phone/fax:
(+48) 22 5720702

Abstract

Introduction: Studies have demonstrated leptin involvement in the physiology and pathophysiology of pregnancy and suggest that leptin may be a prognostic marker for some complications of pregnancy although the association remains unclear. Current studies on leptin concentrations during normal pregnancy lack reference intervals for leptin which could be used for interpreting the differences in leptin levels found in normal and pathological pregnancies.

Objective: To examine leptin concentrations in maternal serum in the third trimester of normal pregnancy and in cord blood and to establish reference intervals for leptin.

Material and methods: The study was performed in 194 normal pregnant women. Leptin concentrations in maternal sera and in cord blood were measured by ELISA and subsequently analysed by gestational age (weeks), maternal Body Mass Index (BMI), mode of delivery and neonatal gender and birth weight.

Results: In normal pregnant women, the mean serum leptin concentration measured at delivery was 37.17 ± 28.07 ng/mL and the established reference interval was 33.19–41.14 ng/mL. The mean leptin concentration in cord blood was 14.78 ± 15.97 ng/mL and the established reference interval was 12.32–17.67 ng/mL. There was a statistically significant positive correlation

Key words:

leptin, serum, pregnancy, cord blood, third trimester

between maternal serum and cord blood leptin concentrations (r= 0.037; p=0.00). Mean leptin concentrations in cord blood increased with gestational age (p=0.00). No statistically significant differences in maternal serum and cord blood leptin concentrations were found in regard to mode of delivery and neonatal gender. A statistically significant correlation was found between maternal serum leptin and third-trimester BMI (r=0.22; p=0.00), but there was no association between maternal BMI and cord blood leptin concentration. There was a statistically significant positive correlation between cord blood leptin concentration and birth weight (r=0.23; p=0.00).

Conclusions: Reference intervals for leptin in maternal serum and in cord blood established in normal pregnancy could be used in clinical practice for interpreting the differences in leptin concentrations found in normal pregnancy and in complications of pregnancy. The results indicate a strong association between maternal serum leptin levels and obesity and between cord blood leptin levels and birth weight.

Introduction

Leptin is an anorexigenic polypeptide hormone containing 167 amino acids, with a molecular weight of 16.7 kDa [1]. It was discovered and first described in 1994 by Zhang et al. as the product of Ob (obesity) gene [2]. Leptin is produced primarily by subcutaneous adipose tissue, but also by the hypophysis, stomach, skeletal muscle, vascular endothelium, brown adipose tissue, brain and placenta [3–5]. Leptin is a pleiotropic hormone regulating several metabolic pathways. It is involved in metabolism and nutrition as well as in immune and endocrine processes [3,6,7]. Blood leptin concentrations are influenced by age, gender and levels of other hormones in the body [8,9]. The production of leptin and its blood concentrations increase in direct proportion to the amount of subcutaneous adipose tissue as confirmed by numerous studies demonstrating a strong association between serum leptin and Body Mass Index (BMI). Leptin levels are higher in obese individuals than in people with normal body weight [10,11]. Serum levels of leptin in women are at their highest during pregnancy [12,13] as a result of increasing body weight and the effect of the placenta and the developing fetus on the production of leptin. Serum levels of leptin increase steadily across pregnancy reaching a peak at approximately 28 weeks to fall dramatically to the pre-pregnancy values within 24 hours postpartum [14,15]. According to some authors, leptin is involved in the regulation of prenatal hematopoiesis and in brain development [16]. High leptin concentrations found in cord blood during childbirth and in neonatal capillary blood soon after birth correlated with birthweight [17]. It has been observed that leptin concentrations are higher in maternal serum than in cord blood due to placental involvement in the metabolism and production of leptin [18,19]. Studies have demonstrated that adequate leptin production is necessary for maintaining a normal pregnancy and proper development of the embryo and fetus [20–22]. It remains to be elucidated, however, whether leptin levels can be used as a predictor of complications of pregnancy. Published studies report changes in serum leptin levels during pregnancy but they lack clearly defined leptin values in normal pregnancy. It may expected that determining leptin concentration ranges in the sera of women identified as having a normal pregnancy and in cord blood could help to establish the reference intervals for leptin in the third trimester to be used in clinical practice for interpreting the differences in leptin concentrations observed in normal and pathological pregnancies.

Material and methods

A total of 194 full-term pregnant women were included in the study. They were identified as having a normal pregnancy and admitted for delivery to two hospitals in Warsaw, Poland, St. Sophia Hospital and the Transfiguration Hospital, in the period between January 2015 and June 2017.

The inclusion criteria were: age ≥18 years, third trimester of a singleton pregnancy identified as normal, subject's consent for blood collection at delivery (samples of maternal venous blood and neonate's cord blood) for subsequent measurement of leptin levels.

The clinical material used in the study consisted of two independent sets of blood samples: maternal serum assessed for leptin expressed by the placenta and cord blood assessed for leptin expressed by the fetal tissues. Maternal blood samples (9 mL) were collected from an antecubital before delivery and fetal blood samples (9 mL) were collected from the umbilical vein immediately after delivery. After collection and centrifugation of full blood, sera were stored at -80°C until leptin measurement. Measurements were performed by the immunoenzymatic test ELISA using commercial kits (R&R System Bio-Techne).

Statistical analysis was carried out using STATISTICA PL (StatSoft, Poland). Descriptive statistics were used to present descriptions of quantitative variables. For comparative analyses of normally distributed variables, parametric tests were used such as the Student t-test to test the assumption of homogeneity or non-homogeneity of variance and a One-Way ANOVA when more than two groups were compared. When the variables were not normally distributed, the non-parametric Mann-Whitney test was used. The Pearson linear correlation coefficient was calculated to assess the correlation between normally distributed variables ($p<0.05$).

The reference intervals for leptin were obtained according to the Clinical and Laboratory Standards Institute (CLSI) guidelines by referring to the central 95% of laboratory test values [23]. The study was approved by the Bioethics Committee at the Medical University of Warsaw.

Results

The mean age of study subjects was 30.0 ± 5.2 years (range: 18–44); the mean third-trimester BMI was 28.2 and the mean gestation at delivery 38.9 weeks. The mean birth weight was 3354 g and birth length 54 cm (Table 1).

The mean maternal serum leptin concentration was 37.17 ± 28.07 ng/mL (range 0.25 ng/mL – 121.2 ng/mL) and the established reference interval was 33.19 ng/mL – 41.14 ng/mL. The mean cord blood leptin concentration was 14.78 ± 15.97 ng/mL (range 0.37 ng/mL – 97.8 ng/mL) and the established reference interval was 12.63 ng/mL – 17.67 ng/mL (Table 2).

The mean concentration of leptin was by 60% lower in cord blood than in maternal blood, the difference being statistically significant at $p = 0.00$.

The lowest mean maternal serum leptin concentration at delivery was measured at 38 weeks gestation and the highest at 37 weeks. Mean cord blood concentrations tended to increase with gestational age at delivery. At 40 weeks, the mean concentration was by 8.42 ng/mL higher than that measured at 37 weeks (Fig. 1) and the difference was statistically significant.

Table 1.
Study group characteristics

	Mean	Min	Max	25th quartile	75th quartile	SD
Maternal age	30.0	18.0	44.0	26.0	34.0	5.2
Third-trimester BMI	28.1	17.5	40.2	25.2	30.7	4.1
Gestation at delivery (weeks)	38.9	37.0	40.0	38.0	40.0	1.1
Birth weight (g)	3454.3	1480.0	5030.0	3190.0	3780.0	503.9
Birth length (cm)	54.9	44.0	62.0	54.0	57.0	2.7

Table 2.
Reference values for leptin concentrations in maternal serum and in cord blood

Leptin concentration	N	Mean	CI -95%	CI 95%	Min	Max	Lower quartile	Upper quartile	SD
Maternal serum (ng/mL)	194	37.17	33.19	41.14	0.25	121.2	14.80	55.27	28.07
Cord blood (ng/mL)	194	14.78	12.52	17.04	0.37	97.8	4.98	18.35	15.97

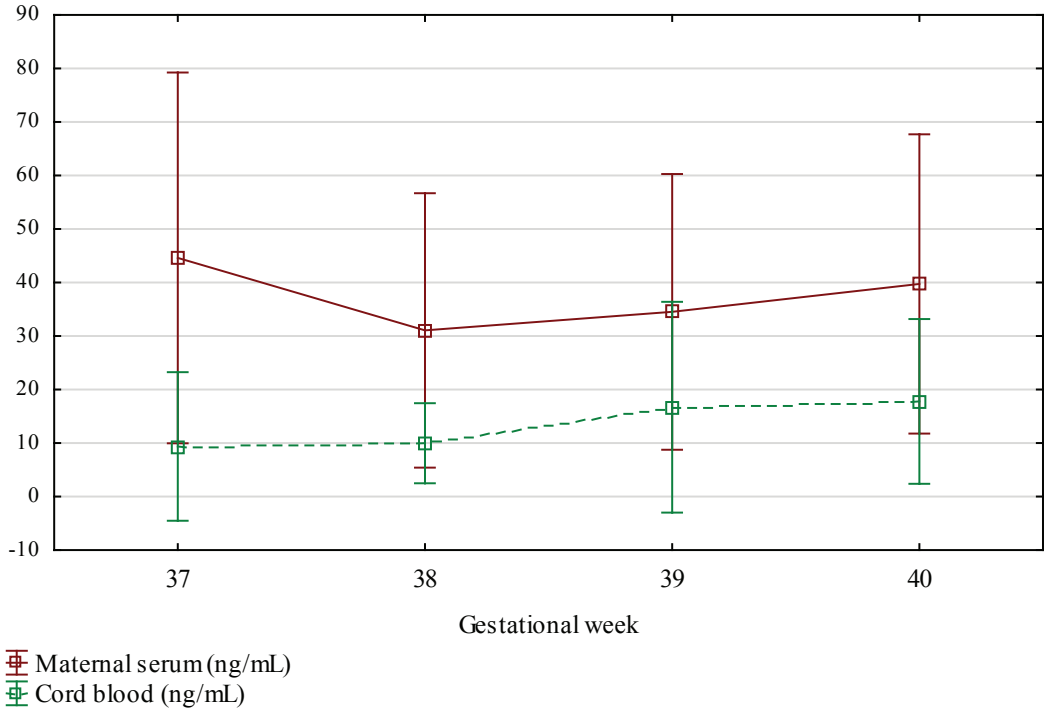

Fig. 1.
Cord blood leptin concentrations by gestational age (week)

No statistically significant differences in maternal serum and cord blood leptin concentrations associated with neonatal gender or mode of delivery were found (Table 3). Leptin concentrations in maternal serum were strongly associated with third-trimester BMI, especially BMI>29. Maternal BMI, however, had no effect on cord blood leptin.

A statistically significant positive correlation was found between maternal serum leptin concentrations and cord blood leptin concentrations ($r = 0.37$; $p = 0.00$). This association was stronger in male neonates ($r = 0.44$); $p = 0.02$) than in female neonates ($r = 0.24$; $p = 0.02$).

The correlation between serum leptin concentration and cord blood concentration was weaker in women who had vaginal delivery ($r = 0.35$; $p = 0.00$) than in those who delivered by Cesarean section ($r = 0.43$; $p = 0.00$), but in both cases the correlation was statistically significant (Fig. 2).

Leptin concentrations were assessed in respect to birth weight and no statistically significant correlation was found between maternal serum leptin concentration and neonatal birth weight.

However, there was a statistically significant positive correlation between cord blood leptin concentration and neonatal birth weight ($r = 0.23$; $p = 0.00$) (Fig. 3). This correlation was significant with non-operative vaginal deliveries ($r = 0.21$; $p = 0.02$), but not with deliveries by Cesarean section ($r=0,22$; $p=0,08$). The correlation between cord blood leptin concentration and neonatal birth weight was stronger in female neonates ($r = 0.25$; $p = 0.02$) than in male neonates ($r = 0.21$; $p = 0.04$) but both correlations were statistically significant.

Table 3.
Maternal serum leptin and cord blood leptin by neonatal gender, mode of delivery, gestational age at delivery and third-trimester BMI

	N	Maternal serum leptin (ng/mL)			Cord blood leptin (ng/mL)		
		Mean	SD	p value	Mean	SD	p value
Neonatal gender							
Female	94	34.28	25.31		13.38	10.82	
Male	100	39.89	30.31	0.16	16.10	19.58	0.24
Mode of delivery							
Vaginal, non-operative	129	37.83	28.04		15.48	15.29	
Cesarean section	65	35.86	28.30	0.65	13.38	17.26	0.39
Gestation at delivery (weeks)							
37	28	44.61	34.64		9.37	19.01	
38	36	31.06	25.63		9.98	7.49	
39	62	34.53	25.76		17.40	22.57	
40	68	39.75	27.96	0.19	17.79	15.39	0.02
Third-trimester BMI							
<25	43	32.41	30.19		13.70	16.22	
<25-29>	77	32.74	25.54		15.31	17.45	
>29	74	44.54	28.12	0.02	14.86	14.30	0.87

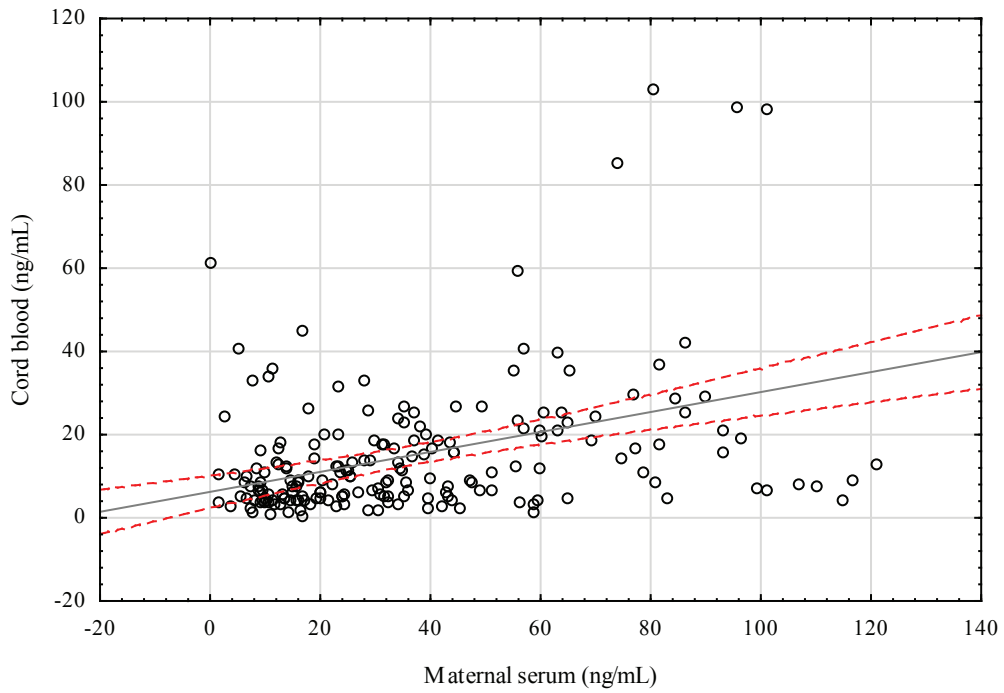

Fig. 2.
Correlation between maternal serum leptin concentration and cord blood leptin concentration

A statistically significant correlation was found between maternal serum leptin concentration and third-trimester BMI (r = 0.22; p = 0.00), but not between cord blood leptin concentration and third-trimester BMI (Fig. 4).

Discussion

We determined mean maternal blood leptin concentrations in the third trimester of a normal pregnancy. The highest leptin level was found at 38 weeks gestation and the lowest at 37 weeks. Published studies have attributed elevated leptin levels during pregnancy to substantial increases in the amount of adipose tissue [2]. The results of our study confirm a correlation between maternal blood leptin and BMI. Other authors believe that increased leptin concentrations in maternal blood are due to the placental production of leptin as documented by assessment of maternal leptin levels during pregnancy and postpartum. Leptin concentrations rise as early as the first

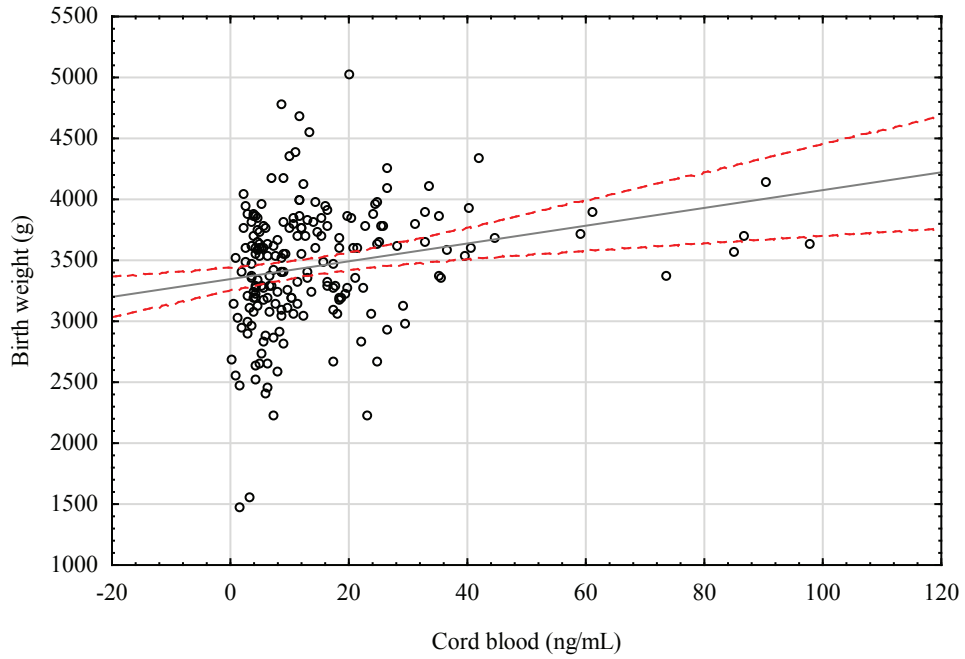

Fig. 3.
Correlation between cord blood leptin concentration and birth weight

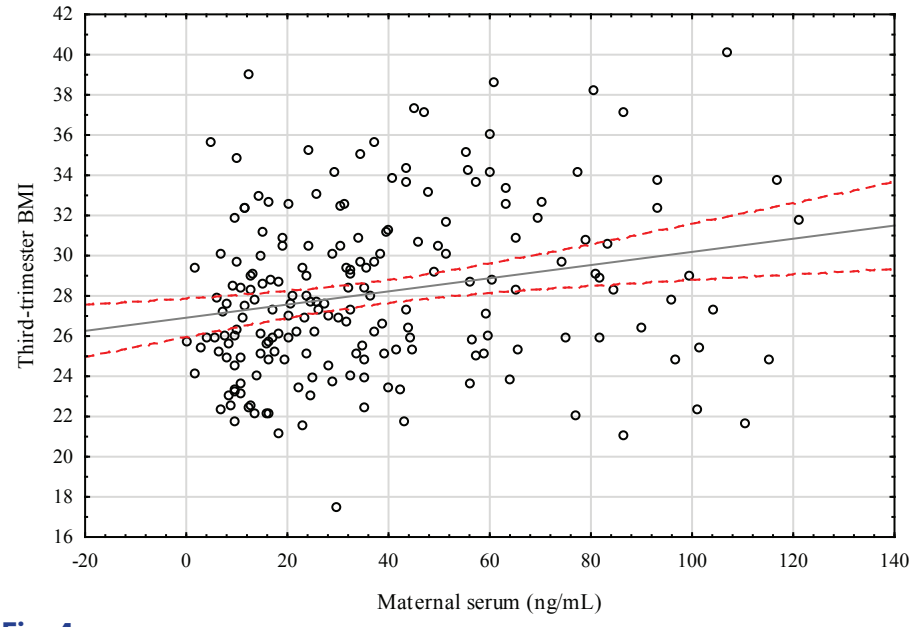

Fig. 4.
Correlation between maternal serum leptin concentration and third-trimester BMI

trimester, when the increase in the amount of adipose tissue is not dramatic and fall to the pre-pregnancy values within 24 hours after delivery [14]. With the two sources of leptin, i.e. adipose tissue and the placenta, leptin concentrations are higher in maternal blood than in cord blood as confirmed by Sagawa et al. [24]. Laml et al. also observed the difference but in their study it was not significant. They hypothesized that leptin, a 16-kDa protein cannot cross the placenta [25]. In our the study, the mean cord blood leptin concentration was nearly twice as low as the mean concentration in maternal serum. We demonstrated a statistically significant positive correlation between the mean maternal serum leptin concentrations in all weeks of gestation at delivery and umbilical cord leptin which may be associated with placental growth during pregnancy. Marino-Ortega et al. observed a similar correlation [26]. We did not find any differences in leptin levels, both maternal and neonatal, associated with mode of delivery. The available literature data are not consistent. Yoshimitsu et al. found statistically higher cord blood leptin concentrations in neonates born by vaginal delivery [27]. The differences were accounted for by augmented synthesis and release of leptin due to decreased oxygenation of blood during vaginal delivery with activation of the sympathetic system and the resulting increase in cortisol synthesis which stimulates leptin release. The above results were not confirmed by other authors, including a study in women with gestational diabetes but otherwise normal pregnancy [28]. A number of studies have shown that leptin levels are higher in women who have more subcutaneous adipose tissue than men while estrogen stimulates leptin release by adipocytes and acts on the hypothalamus increasing its leptin sensitivity [24,29]. We did not observe any significant differences in leptin concentrations in either maternal serum or cord blood associated with neonatal gender. When neonatal birth weights and other anthropometric measurements were similar, there were no apparent differences in leptin concentrations between genders. Some authors have observed higher cord blood leptin concentrations in female neonates and although the cause has not been convincingly explained, estrogen has been implicated as possibly increasing leptin levels in female infants [30–32]. These inconsistent findings may result from studies based a single leptin measurement with no measurements of other hormones involved in the regulation of leptin levels. We also assessed leptin levels in relation to neonatal birth weight and found no statistically significant association with maternal serum leptin which confirms the results obtained by other authors [19,33,34]. However, there was a statistically significant correlation between cord blood leptin and neonatal birth weight. This finding is confirmed by other authors who underlie the role of leptin in the regulation of appetite and metabolism, which may also lead to changes in body weight in the first days after birth and suggest that leptin could be a marker of nutritional status [28,35–40]. A significant correlation was found between maternal BMI and maternal serum leptin concentration, but not cord blood leptin. Marino-Ortega et al. reported similar findings of a strong association of maternal leptin blood levels with weight gain during pregnancy [26]. Shroff et al. showed a moderate correlation between maternal leptin levels and BMI [41]. These inconsistent observations in pregnant women may suggest that the association between maternal BMI and leptin levels is not obvious and other factors or complications of pregnancy may affect leptin levels. Further studies are needed to elucidate leptin involvement in some complications of pregnancy and to evaluate potential uses of leptin measurements as a predictor of pathological pregnancy. First, a reference interval should be obtained for leptin levels in normal pregnancy, followed by establishing week-by-week reference intervals in pregnancies identified as pathological. Comparison of these sets of leptin values would be helpful in correct assessment of leptin measurements in pregnancy and could potentially lead to better understanding of the role of leptin in the pathomechanism(s) of some disorders of pregnancy or even to the use of serum leptin as a biomarker for such disorders.

Conclusions

1. The study established the reference intervals for leptin concentrations in maternal serum

and cord blood in the third trimester of normal pregnancy, according to the Clinical and Laboratory Standards Institute (CLSI) guidelines.

2. The reference intervals for leptin concentrations in maternal serum and in cord blood could be potentially used in clinical practice to assess leptin measurements as suggestive of either normal or pathological pregnancy.
3. Mean third-trimester cord blood leptin concentrations increase with gestational age at birth.
4. There is no association between maternal serum leptin or cord blood leptin concentrations and neonatal gender or mode of delivery.
5. There is a strong correlation between third-trimester BMI and maternal serum leptin concentration.

References

1. Barash IA, Cheung CC, Weigle DS. Leptin is a metabolic signal to the reproductive system. Endocrinology. 1996; 137(7): 3144-3147.
2. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homologue. Nature. 1994; 1:372(6505):425-432.
3. Park HK, Ahima RS. Leptin signaling. F1000Prime Rep. 2014; 4:6:73.
4. Shufen Li, Xi Li. Leptin in normal physiology and leptin resistance. Sci Bull. 2016; 61(19):1480-1488.
5. Hegyi K, Fulop K, Kovacs K et al. Leptin induced signal transduction pathways. Cell Biol Int. 2004; 28(3):159-169.
6. Perez-Perez A, Sanchez-Jimenez F, Maymo J et al. Role of leptin in female reproduction. Clin Chem Lab Med. 2015; 53(1):15-28.
7. Behnes M, Brueckmann M, Lang S et al. Alterations of leptin in the course of inflammation and severe sepsis. BMC Infect Dis. 2012; 12:217.
8. Auwerx J, Steals B. Leptin. Lancet. 1998; 351:737-742.
9. Considine RV, Sinha MK, Heiman MI et al. Serum immunoreactive – leptin concentrations in normal – weight and obese humans. N Eng J Med. 1996; 1:334(5):292-295.
10. Bernotiene E, Palmer G, Gabay C. The role of leptin in innate and adaptive immune responses. Arthritis Res Ther. 2006; 8(5):217-227.
11. Schwartz MW, Peskind E, Raskind M, Boyko EJ, Porte DJ. Cerebrospinal fluid leptin levels: relationship to plasma levels and to adiposity in humans. Nat Med. 1996; 2(5):589-593.
12. Wauters M, Considine RV, Van Gaal LF. Human leptin: from an adipocyte hormone to an endocrine mediator. Eur J Endocrinol. 2000; 1:43(3):293-311.
13. Chien EK, Hara M, Rouard M et al. Increase in serum leptin and uterine leptin receptor messenger RNA levels during pregnancy in rats. Biochem Biophys Res Commun. 1997; 18:237(2):476-480.
14. Masuzaki H, Ogawa Y, Sagawa N et al. Nonadipose tissue production of leptin: leptin as a novel placenta-derived hormone in humans. Nat Med. 1997; 3(9):1029-1033.
15. Henson MC, Castracane VD. Leptin in pregnancy: an update. Biol Reprod. 2006; 74(2):218-229.
16. Turner RT, Philbrick KA, Kuah AF et al. Role of estrogen receptor signaling in skeletal response to leptin in female ob/ob mice. J Endocrinol. 2017; 233(3):357-367.
17. Ashima RS, Yong Qi, Singhal NS et al. Brain adipocytokine action and metabolic regulation. Diabetes. 2006; 55(2):145-154.
18. Ashima RS, Lazar MA. Adipocytokines and the peripheral and neutral control of energy balance. Mol Endocrinol. 2008; 22(5):1023-1031.
19. Tamura T, Goldenberg RL, Johnston KE, Cliver SP. Serum leptin concentrations during pregnancy and their relationship to fetal growth. Obstet Gynecol. 1998; 91(3):389-395.
20. Pérez-Pérez A, Toro A, Vilariño-García T et al. Leptin action in normal and pathological pregnancies. J Cell Mol Med. 2018; 22(2):716-727.
21. Weiss G, Sundl M, Glasner A, Huppertz B, Moser G. The trophoblast plug during early pregnancy: a deeper insight. Histochem Cell Biol. 2016; 146(6):749-756.
22. Sheikh AM, Small HY, Currie G, Delles C. Systematic review of micro-RNA expression in pre-eclampsia identifies a number of common pathways associated with the disease. PLoS ONE. 2016; 16;11(8):e0160808.
23. Horovitz GL, Altaie S, Boyd JC i wsp. Defining, Establishing and Verifying Reference Intervals in the

Clinical Laboratory; Approvied Guideline-Third Edition. Clinical and Laboratory Standard Institute. 2010; (28):30.

24. Sagawa N, Yura S, Itoh H et al. Role of leptin in pregnancy - a review. Placenta. 2002; 23:80-86.
25. Laml T, Hartmann BW, Ruecklinger E, Preyer O, Soeregi G, Wagenbichler P. Maternal Serum Leptin Concentrations Do Not Correlate With Cord Blood Leptin Concentrations in Normal Pregnancy. J Soc Gynecol Investig. 2001; 8(1):43-47.
26. Marino-Ortega LA, Molina-Bello A, Polanco-García JC et al. Correlation of leptin and soluble leptin receptor levels with anthropometric parameters in mother-newborn pairs. Int J Clin Exp Med. 2015; 15:8(7):11260-11267.
27. Yoshimitsu N, Douchi T, Kamio M, Nagata Y. Differences in umbilical venous and arterial leptin levels by mode of delivery. Obstet Gynecol. 2000; 96(3):342-345.
28. Bury A, Kulik-Rechberger B. Relationships Between Leptin Concentration and Sex, Type of Delivery and Body Weight of Newborns During First Days of Life. Endokrynol Ped. 2011; 10(3):29-38.
29. Matsuda J, Yokota I, Iida M. Dynamic changes in serum leptin concentrations during the fetal and neonatal periods; Pediatr Res. 1999; 45(1):71-75.
30. Watanobe H, Habu S. Manipulation of neonatal gonadal steroid milieu and leptin secretion in later life in male and female rats. Regul Pept. 2003; 110(3):219-224.
31. Schubring C, Siebler T, Kratzch J et al. Leptin serum concentrations in healthy neonates within the first week of life: relation to insulin and growth hormone levels, skinfold thickness, body mass index and weight. Clin Endocrinol (Oxf). 1999; 51(2):199-204.
32. Mellati AA, Mazloomzadeh S, Anjomshoaa A et al. Multiple correlations between cord blood leptin concentration and indices of neonatal growth. Arch Med Res. 2010; 41(1):26-32.
33. Butte NF, Hopkinson JM, Nicolson MA. Leptin in human reproduction: serum leptin levels in pregnant and lactating women. J Clin Endocrinol Metab 1997; 82: 1480–1483.
34. Schubring C, Kiess W, Englaro P et al. Levels of leptin in maternal serum, amniotic fluid and arterial and venous cord blood: relation to neonatal and placental weight. J Clin End Metab 1997; 82:480–483.
35. Catov JM, Patrick TE, Powers RW, Ness RB, Harger G, Roberts JM. Maternal leptin across pregnancy in women with small-for-gestational-age infants. Am J Obstet Gynecol. 2007; 196(6):558.e1-8.
36. Kirel B, Tekin N, Tekin B, Kiliç FS, Doğruel N, Aydoğdu SD. Cord blood leptin levels: relationship to body weight, body mass index, sex and insulin and cortisol levels of maternal-newborn pairs at delivery. J Pediatr Endocrinol Metab. 2000; 13(1):71-77.
37. Su PH, Wang SL, Chen JY, Lai CP, Jian SH. Serum leptin levels in preterm, healthy and sick-term newborns. Acta Paediatr Taiwan. 2002; 43(5):249-254.
38. Garanty-Bogacka B, Czeszyńska MB, Syrenicz M. Immaturity or hypotrophy? The cord blood leptin levels in preterm and small for gestational age neonates. Gin Pol. 2003; 74:356-361.
39. Po-Jung Tsai, Chun-Hsien Yu, Shih-Penn Hsu; Cord plasma concentrations of adiponectin and leptin in healthy term neonates: positive correlation with birthweight and neonatal adiposity. Clin Endocrinol (Oxf). 2004; 61(1):88-93.
40. Valūniene M, Verkauskiene R, Boguszewski M et al. Leptin levels at birth and in early postnatal life in small- and appropriate-for-gestational-age infants. Medicina (Kaunas). 2007; 43(10):784-791.
41. Shroff MR, Holzman C, Tian Y, Evans RW, Sikorskii A. Mid-pregnancy maternal leptin levels, birthweight for gestational age and preterm delivery. Clin Endocrinol (Oxf). 2013; 78(4):607-613.