Predictors of Cord Blood Leptin Level in Pregnancies Complicated With Preeclampsia, Fetal Growth Restriction and in Normal Pregnancies¶

Nilgün ÖZTÜRK DOĞAN, Serdar YALVAÇ, Eray ÇALIŞKAN, Asuman ERTEN, İsmail DÖLEN, Ali HABERAL
Ankara- Turkey

OBJECTIVE: To investigate possible predictors of cord blood leptin level in pregnancies complicated with fetal growth restriction, preeclampsia and in normal pregnancies.
STUDY DESIGN: Thirty-two pregnant women with intrauterine growth restriction (IUGR), 28 women with preeclampsia and randomly selected 36 normal pregnant women were enrolled. Umbilical cord blood samples were collected for measurements of leptin, cortisol and blood gas after delivery.
RESULTS: Cord blood leptin level was significantly lower in the IUGR group (8.9±9ng/ml, p=0.01) compared to control group (18.8±15.8) but not different from preeclampsia group (14.8±15.8). Cord blood cortisol level was significantly higher in the preeclampsia group (27.8±13.4 Ug/dl, p=0.005) compared to control group (19.5±9.2) and IUGR group (18.6±12.7). Multiple lineer regression model revealed that fetal birth weight, fetal gender, gestational age, maternal hematocrite, cord blood cortisol level, cord blood hematocrite and presence of fetal intrauterine growth restriction were all independent predictors of cord blood leptin level.
CONCLUSION: Regulation of cord blood leptin level is a complex process involving fetal gender and fetal anthropometric variables as well as cord blood cortisol, intrauterine growth and hypoxia. Leptin level is decreased in cases of placental insufficiency like IUGR but not in uncomplicated preeclampsia alone.
(Gynecol Obstet Reprod Med 2007;13:1 14-20)

Key Words: Fetal leptin, Predictors, Fetal growth, Cortisol, Hematocrite, Preeclampsia

The regulation of fetal growth during gestation remains poorly understood. In recent years, considerable interest has developed concerning the role of leptin in the regulation of fetal nutrient uptake and growth. It is a hormone secreted mainly by adipocytes1 and acts on the hypothalamic centers to regulate body weight,[2] decrease food intake and increase body temperature and energy expenditure.[3]

During human pregnancy, maternal leptin concentration peak during the second trimester and remain elevated until parturition. However, maternal leptin concentrations may not be an accurate indicator of fetal growth because they do not correlate directly with birth weight.[4] On the other hand cord blood leptin concentrations obtained at delivery was found to be correlated with birth weight.[2] It has been suggested that fetal fat mass is a major determinant of fetal circulating leptin levels and that leptin might be involved in the regulation of fetal growth.[5,6]

Department of Obstetrics and Gynecology, T.R. Ministery of Health Ankara Etlik Maternity and Women's Health Teaching and Research Hospital, Ankara

Address of Correspondence *Nilgün Öztürk Doğan*
Fevzi Çakmak Sok. 10/13
Murat Apt.
06440 Kızılay - Ankara

Submitted for Publication: 26.12.2006
Accepted for Publication: 19.03.2007

¶ *This manuscript has been presented as a poster in the 6th International Turkish-German Gynecological Association Congress (19-22 May 2005 Antalya-Turkey)*

Birth weight and placental weight were found to be positively correlated with umbilical cord blood leptin level,[7] which has independent association with intrauterine growth restriction and macrosomia.[6] Maternal serum leptin level was drastically reduced after placental delivery.[8] but there was no correlation between maternal and cord blood leptin levels.[9] These findings suggest that maternal and cord blood leptin levels are regulated by different mechanisms.

Preeclampsia increases fetal risk of being born small for gestational age.[10] The growth-retarded infants exhibit wasting of subcutaneous fat, and one would expect lower leptin levels in umbilical blood obtained from preeclamptic compared to normotensive pregnancies.[10] Despite this in dependent effect of preeclampsia on fetal cord blood leptin level in cases with and without intrauterine growth restriction has not been adequately investigated.

The aim of the present study is to investigate the possible predictors of cord blood leptin level in pregnancies with fetal growth restriction, preeclampsia and normal pregnancies.

Materials And Methods

The study was performed at the Ankara Etlik Maternity and Women's Health Teaching and Research Hospital between 01-September-2002 and 28-February-2003. Thirty-two consecutive pregnant women with intrauterine growth restriction, 28 consecutive women with preeclampsia and 36 healthy pregnant women (control group) were enrolled in the study. Preeclamptic women with growth restricted fetuses

were included in intrauterine growth restriction group. Control group consisted of the first consequent women delivering after intrauterine growth restricted or preeclamptic women who gave consent. The Institutional Review Board and the Research Committee for Human Subjects approved the study. Written informed consent was obtained from all subjects before enrollment in the study.

Demographic and obstetric histories of the mothers were obtained at admittance. Maternal body mass index (BMI) was calculated using standard tables, and was defined as weight (in kilograms) divided by height (in squared meters). Blood and urine were collected to measure complete blood count, liver function tests, renal function tests, urine analysis and fasting glucose at admittance to the labor ward.

Preeclampsia was defined as, persistent diastolic blood pressure of at least 90 mmHg after 20 weeks of gestation, and diastolic blood pressure increase by at least 25 mmHg measured within six hours intervals. In addition, proteinuria cut-off value was defined as 300 mg or more of protein collected in 24 hour urine specimen after 20 weeks of gestation without any urinary infection or known renal disease.

The presence of gestational diabetes mellitus or fasting blood glucose upper than 105 mg/dl at admission, were exclusion criteria according to National Diabetes Data Group (NDDG-1979). Gestational diabetes mellitus was routinely ruled out by normal oral glucose tolerance test results (World Health Organisation protocol) between 24 to 28 weeks of gestation.[11] Other exclusion criteria were: multiple pregnancy, systemic maternal infection, premature rupture of membranes, placental abruption, liver and renal disease.

Serial ultrasound follow-up was undertaken in all fetuses to assess intrauterine growth restriction (IUGR). The diagnosis of fetal growth restriction was made antenatally when the abdominal circumference by ultrasound measurements was below the third percentile of reference values according to Hadlock formula. Umbilical artery and if necessary middle cerebral artery Doppler measurements were performed in IUGR fetuses; S/D values were taken pathological for umbilical artery Doppler measurements according to normograms of gestational weeks. Abnormal umbilical artery Doppler measurements were defined as high umbilical artery S/D ratio, absent or reverse end diastolic flow in the umbilical artery in all cases. All ultrasonographic measurements were performed by the same operator with the use of an ATL 3000 Advanced with a 5 MHz transabdominal C4-2 convex probe. The period elapsed between the final ultrasonography and births, was maximum 7 days.

Continuous fetal heart rate monitoring (Space Lab antepartum monitor 67) was done in all cases during labour and if there was an obstetric indication, cesarean section was performed. Intrapartum fetal heart rate (FHR) patterns were classified according to Kubli et al.[12] as a consensus of any of the two available investigators. Apgar scores at 5th minute, neonatal birth weight and length, placental weight, the mode of delivery and neonatal gender were recorded. After separation of the placenta and removal of the attached blood clots, the placenta was weighed separately.

Umbilical cord venous blood samples for leptin, cortisol and arterial samples for blood gas were collected immediately after delivery, in preheparinized plastic syringes. The samples were immediately centrifuged at 2000 rpm for 15 minutes and all serum samples were stored at –20 ^{o}C until assayed within a month. Serum leptin levels were measured by radioimmunoassay (Active® Human Leptin IRMA; DSL-23100). All samples were analysed in duplicate, and the assay has a sensitivity of 0.01 ng/mL. Intra-assay coefficient variation was less than 5.2%, and the interassay variation was 8.7% for leptin values. Serum cortisol levels were measured by radioimmunoassay (Active® Cortisol RIA; DSL-2100). All samples were run in duplicate, and the detection of the assay limit was 0.01 Ug/dL. Intra-assay and interassay coefficient variation was 2.4% and 5.8%, respectively. Blood gas analysis was performed using IL-Synthesis System.

All statistical analyses were performed using the Statistical Package for the Social Science (SPSS version 10.0, Inc. Chicago, III). Shapiro-Wilk normality tests were performed for the distribution of continuous data. If the distribution was normal, ANOVA and Tukey tests were used for the comparison of continuous variables between the three groups, if not Kruskal-Wallis test was used. The distribution of the frequency variables according to the groups was analyzed by Chi-square test or likelihood tests whenever suitable. The presence of a correlation between the variables was analyzed by Pearson correlation analysis. Multivariate lineer regression analysis with stepwise method was used to identify the independent predictors of leptin levels as the dependent continuous variable. Predictors of leptin level explored in this analysis included maternal age, mean arterial pressure, fasting glucose level, body mass index, presence or absence of preeclampsia, IUGR, tobacco smoking, maternal hematocrite level, oligohydramnios, meconium stained amniotic fluid, non-reassuring fetal heart rate tracings, gestational age (days) as confirmed with second trimester ultrasound measurements, mode of delivery (vaginal vs cesarean), fetal birth weight, placental weight, fetal gender, cord blood pH, base excess, cord blood hematocrite and cortisol. For all comparisons probability $p<0.05$ was considered to be statistically significant.

Results

Demographic and obstetric characteristics of the maternal study population are presented in Table 1.

Table 1. Demographic characteristics of the maternal study population. Data are presented as mean ± standart deviation or numbers and percentages.

Variables	IUGR group (n=32)	Preeclampsia group (n=28)	Control group (n=36)	P
Maternal age (years)	25.7 ± 4.8	28.6 ±6.7	24.4 ± 3.5	0.05*
Maternal weight (kg)	69.7 ±8.9	75.4 ±12.8	71.1 ± 11.6	0.1**
Maternal height (cm)	157.9 ± 6.3	158.3 ±6.1	159.5 ± 5.2	0.5**
Maternal BMI (kg/m^2)	27.9 ±3.2	30 ± 4.8	28.2 ± 4.4	0.1**
Gravidity	2 ± 1.3	2.3 ± 1.4	1.8 ± 0.8	0.2**
Parity ≥1	13 (40.6)	14 (50)	20 (55.6)	0.4***
Abortion ≥1	6 (18.7)	3 (10.7)	4 (11.1)	0.5***
Dilatation and curettage ≥1	0	2 (7.2)	2 (5.6)	0.3***
Intrauterin fetal death≥1	4 (12.5)	2 (7.1)	2 (5.6)	0.5***
Tobacco use	7 (21.9)	3 (10.7)	6 (16.7)	0.5***
Time since last pregnancy (months)	45.7 ± 45.1	50 ± 31	38.2 ± 31.4	0.5*

** Not statistically significant, Kruskal-Wallis test (p>0.05)*
*** Not statistically significant, ANOVA test (p>0.05)*
**** Not statistically significant, Chi-square test (p>0.05)*

There was no statistically significant difference between groups according to maternal age, maternal weight and height, maternal BMI, gravidity, number of live birth, number of abortion, number of dilatation and curettage, number of intrauterine fetal death, tobacco use and time elapsed since last pregnancy.

Maternal clinical characteristics according to groups at admission are presented in Table 2.

between control group and the two others. Gestational age estimated by the last menstrual period and via ultrasound measurements done at the second trimester were significantly lower in the IUGR group than the other groups (p<0.001). Oligohydramnios and abnormal umbilical artery Doppler measurements were more frequent in the IUGR group (p<0.001 and p<0.001 respectively).

The distribution of intrapartum variables according to the

Table 2. Maternal and fetal variables at admission. Data are presented as mean ± standard deviation or numbers and percentages.

Variables	IUGR group (n=32)	Preeclampsia group (n=28)	Control group (n=36)	P
Hypertension	9 (28.1)	28 (100)	0	<0.001*
Antihypertensive medication	8 (25)	5 (17.9)	0	0.008*
Mean arterial pressure at admittance (mmHg)	98.6 ± 19.5	116.9 ± 11.2	85.1 ± 7.1	<0.001*
Hematocrite (%)	36.1 ± 3.2	34.8 ± 4.9	36.4 ± 3.4	0.2
Fasting glucose (mg/dl)	90.3 ± 9.4	92.7 ± 9.9	91.4 ± 8	0.5
Gestational age by last menstrual period (days)	259.5 ± 14.7	275 ± 9.8	277 ± 8.4	<0.001**
Ultrasound estimated gestational age (days)	228.5 ± 11.2	268 ± 8.8	270 ± 8.4	<0.001**
Oligohydramnios	24 (75)	4 (14.3)	0	<0.001*
Abnormal umbilical artery Doppler measurements	21 (65.6)	0	0	<0.001*

** Statistically significant, chi square test, (p <0.05)*
*** IUGR group is significantly different than preeclampsia group (p<0.05) and control group (p<0.05), ANOVA and Tukey tests.*

In accordance with the selection criteria of the study, there was statistically significant difference in the rate of women with hypertension, receiving antihypertensive medication (alpha-methyldopa) and mean blood pressure at admittance

study groups are given in Table 3. The duration of active labour time, the rate of meconium stained amniotic fluid and fetal gender distribution was similar in three groups. On the other hand, the rate of nonreassuring fetal heart rate tracings,

cesarean delivery, low Apgar scores at 5th minute and neonatal intensive care unit admission was significantly more frequent in the IUGR group compared to the other two groups. Birth weight, birth height and placental weight was significantly lower in the IUGR group when compared to preeclampsia and control groups.

Cord blood pH level and chloride level were significantly lower while anion gap and hematocrite levels were significantly higher in IUGR and preeclampsia groups compared to the control group. Cord blood partial oxygen and carbon dioxide pressure were similar among the three groups, while base excess and bicarbonate levels were significantly lower in the

Table 3. Distribution of intrapartum variables according to the study groups. Data are presented as mean ± standart deviation or numbers and percentages.

Variables		IUGR group (n=32)	Preeclampsia group (n=28)	Control group (n=36)	P
Active labour time (min)		263 ± 81	292 ± 127	266 ± 136	0.7
Meconium stained amniotic fluid		1 (%3.1)	2 (%7.1)	0	0.2
Nonreassuring fetal heart rate tracing		15 (%46.9)	2 (%7.1)	3 (%8.3)	<0.001*
Non-reactive		7 (%21.9)	1 (%3.6)	3 (%8.3)	<0.001*
Decelerative		8 (%25)	0	0	<0.001*
Variable deceleration		0	1 (%3.6)	0	0.3
Cesarean section		23 (%71.9)	7 (%25)	17 (%47.2)	0.001*
Apgar score <7 at fifth minute		6 (%18.8)	0	0	0.002*
Birth weight (g)		1942 ± 418	3212 ± 534	3313 ± 385	<0.001**
Birth height (cm)		40 ± 6.2	49 ± 5.7	50.7 ± 2.9	<0.001**
Placental weight (g)		343.5 ± 135.9	618.2 ± 165.5	569.4 ± 106.4	<0.001**
NICU admission		16 (%50)	0	0	<0.001**
Fetal gender	Female	14 (%43.8)	12 (%42.9)	22 (%61.1)	0.2
	Male	18 (%56.3)	16 (%57.1)	14 (%38.9)	

NICU: Neonatal intensive care unit
** Statistically significant, chi square test, (p <0.05)*
*** IUGR group is significantly different than preeclampsia group (p<0.05) and control group (p<0.05), ANOVA and Tukey tests.*

Distribution of fetal cord blood variables according to the study groups are given in Table 4.

preeclampsia group compared to the control group. Cord blood cortisol levels were significantly higher in the

Table 4. Distribution of fetal cord blood variables according to the study groups.

Variables in the Cord Blood	IUGR group (n=32)	Preeclampsia group (n=28)	Control group (n=36)	P
pH level	7.3 ± 0.7	7.3 ± 0.7	7.3 ± 0.4	0.01*
Anion gap (mEq/L)	16 ± 2.7	16 ± 3.8	12.2 ± 2.4	<0.001*
Base excess (mEq/L)	-1.2 ± 2.6	-2.9 ± 3.6	-0.5 ± 1.4	0.003**
Partial O2 pressure (mmHg)	19.3 ± 5.7	21.3 ± 6.6	22.8 ± 7.3	0.1
Partial CO2 pressure (mmHg)	46.2 ± 7.8	44.8 ± 6.6	42.9 ± 6.5	0.2
Bicarbonate (mM/L)	24.6 ± 2.5	22.8 ± 3.2	24.4 ± 1.8	0.02**
Chloride (mEq/L)	102.6 ± 2.4	103.9 ± 2.3	105.6 ± 1.9	<0.001*
Hematocrite (%)	56.3 ± 11.4	57.1 ± 5.3	51.8 ± 7.8	0.03**
Cortisol levels (Ug/dL)	18.6 ± 12.7	27.8 ± 13.4	19.5 ± 9.2	0.005**
Leptin levels (ng/mL)	8.9 ± 9	14.8 ± 15.8	18.8 ± 15.8	0.01***

** IUGR group and preeclampsia group are significantly different (p<0.05) than the control group, ANOVA and Tukey tests.*
*** Preeclampsia group is significantly different (p<0.05) than the control group, ANOVA and Tukey tests.*
**** IUGR group is significantly different (p<0.05) than the control group, ANOVA and Tukey tests.*

preeclampsia group compared to the IUGR and control groups. Cord blood leptin levels were significantly lower in the IUGR group compared to the preeclampsia and control groups.

Multiple lineer regression model revealed that fetal birth weight, fetal gender, gestational age, maternal hematocrite, cord blood cortisol level, cord blood hematocrite and presence of fetal intrauterine growth restriction were all independent predictors of cord blood leptin level (Table 5).

Table 5. Multiple Linear Regression Models of predictors of leptin level. (R= 0.71).

Predictor	β	Standart Error	p	95% Confidence Interval for β
Fetal birth weight (g)	0.01	0.003	<0.001	0.01 – 0.02
Fetal gender male	-10.9	2.35	<0.001	(-15.6) – (-6.3)
Gestational age (days)	-0.43	0.12	0.001	(-0.69) – (-0.18)
Maternal hematocrite (%)	-0.72	0.31	0.02	(-1.34) – (-0.095)
Cord blood cortisol level (Ug/dL)	-0.26	0.01	0.008	(-0.46) – (-0.07)
Cord blood hematocrite (%)	0.28	0.13	0.02	0.03 – 0.54
Fetal IUGR	9.3	4.3	0.03	0.7 – 18

Discussion

Leptin has recently emerged as a potential factor involved in fetal growth that reflects fat mass in newborns.[13] Placental leptin production has been clearly demonstrated inutero.[14] These findings led us to investigate the leptin levels in preeclampsia and IUGR as a potential marker of placental insufficiency.[15] We found that fetal birth weight, fetal gender, gestational age, maternal hematocrite, cord blood cortisol level, cord blood hematocrite and presence of fetal intrauterine growth restriction were all independent predictors of cord blood leptin level.

The positive correlation between cord blood leptin and neonatal birth weight and fetal fat mass was an accepted statement by lots of researchers.[5,6,14,16,17] The variable relationship between neonatal fat mass and birth weight suggests that the relation between birth weight and cord blood leptin depends on the percent of fat mass at birth. These data support the hypothesis that the level of leptin in the fetal circulation is an index of fetal fat mass, similar to the correlation between leptin and various indices of fat mass in children and adults.[9]

Because fetuses affected by growth restriction and small for gestational age newborns have significantly reduced fat tissue accumulation, it has been hypothesized that lower leptin levels in umbilical cord found in these fetuses were determined by the limited amount of fetal fat tissue. In our study, cord blood leptin was found significantly decreased in the intrauterine growth restricted group. However, determination of leptin concentrations in umbilical cord blood of growth restricted fetuses has shown discrepancies, with studies reporting lower[14,18-20] or higher[21] levels compared with normal fetuses. This might be owing to the role played by fetal oxygenation and acid-base balance, because it has been observed that growth restricted fetuses with signs of severe fetal distress have significantly higher leptin concentrations per kilogram of fetal weight than normal fetuses.[22]

Despite the finding that cord blood leptin level is decreased due to decreased fat mass; Takahashi et al., contrary to our study, found increased cord blood leptin level in severe IUGR babies.[23] They stated that intrauterine growth restriction induces chronic and acute asphyxic conditions via the stress labour (contraction stress), causing an alteration in umbilical cord leptin levels.[23] They suggest that when a fetus undergoes stress, free fatty acids may be secreted as a second energy source to compensate for increased glucose consumption, and leptin may have some relation with this mechanism.[23] Thus it seems likely that leptin may have two important roles related to each other: primarily as an acute stress-related hormone, and secondarily for fetal fat mass control.

Similarly, in contrast to what has been observed in the human, Buchbinder et al. found that fetal ovine leptin concentrations were inversely correlated with fetal and placental size and were significantly elevated in the growth restricted ovine fetuses rather than decreased as seen in humans.[24] The ovine growth-restricted fetuses that were used in Buchbinder et al.'s study, had all significant changes in the placental mass and placental vasculature. Chronic reduction in utero-placental blood flow results in significant reduction in umbilical blood flow. In the study of Buchbinder et al., leptin concentrations in the IUGR ovine fetuses were elevated and they concluded that the increased leptin concentrations may be an adaptive response to the decreased uterine and umbilical blood flow that is associated with the IUGR.[24] The reason of low leptin concentrations in IUGR fetuses of the present study may be due to the approach undertaken: when elevated umbilical artery Doppler measurements were detected, the pregnancy was terminated via induction or cesarean section without waiting for the findings to worsen. In our study, it is reasonable

that cord blood leptin level measured, reflects the fetal mass and chronic compensated hypoxia but not the placental leptin, which is stimulated by an acute stress or decompensated chronic hypoxia. In accordance with this, in vitro data indicates that a significant increase in leptin secretion does not occur until 72 hours of acute hypoxia.[25] Our fetal growth restricted population showed no sign of severe fetal distress compared to the control group. It is therefore possible that umbilical cord blood leptin levels depend more on adipose tissue and on fetal oxygenation status rather than on other antenatal development factors. In this study we confirmed that growth restricted fetuses at term show umbilical cord blood leptin concentration significantly lower than those in normal fetuses, indicating that fetal adipose tissue is a major source of leptin.

There is gender difference in adults, with females having higher concentrations of circulating leptin than males.[17,26,27] Our data showed higher cord blood leptin concentrations in female newborns than in male; and multiple logistic regression analysis revealed that gender is one of the independent factors influencing circulating cord blood leptin concentration. According to the strong correlation between cord blood leptin and neonatal fat mass,[13] the higher leptin concentrations in female newborns might be attributed to greater fat mass in female neonates.

Increased cord blood hematocrite levels, anion gap and cord blood CO2 partial pressure and decreased cord blood oxygen partial pressure and base excess in IUGR and preeclamptic mothers' babies of this study, suggest a chronic compensated hypoxia. The increase of cord blood hematocrite was probably an adaptation to this chronic hypoxia. Exposure to hypoxia results in a dramatic decrease in weight gain in the neonates through adult rodent.[28] Plasma leptin levels decrease in association with decreased body weight in newborn and juvenile rats exposed to hypoxia.[28] It can be stated that chronic compensated hypoxia decreases cord blood leptin levels which, operates different than decompansated chronic hypoxia or acute hypoxia.

This is also supported by our findings that preeclampsia is not an independent predictor of leptin level alone. Although leptin level tended to decrease in cases of preeclampsia, other indicators of intrauterine hypoxia like increased IUGR, increased cord blood hematocrite and cord blood cortisol levels were better predictors of cord blood leptin level after adjusting for other confounding variables. Multiple linear regression analysis for predictors of leptin level revealed that cord blood hematocrite was found positively correlated with leptin level, which indicates a compensated hypoxia. We also found that maternal hematocrite, negatively correlated with cord blood leptin levels. This can be explained by the increase of maternal plasma viscosity and vasoconstriction in plasental capillaries which leads to a placental insufficiency in preeclamptic and IUGR gestations. This may also be a consequence of inaapropriate maternal plasma expansion in preeclamptic pregnancies.

Several authors have reported higher cord leptin levels in preeclamptics as compared to normal pregnant women,[29] which was not the case in the presented study. One could hypothesize that cord blood leptin level in preeclampsia could depend on the severity of the disease and the degree and onset of hypoxia.

Leptin has probably a major role in the intrauterin, intrapartum and neonatal period of an infant, in relation with cortisol, for the adaptation to stress, hypoxia and the energy expenditure. The strong correlation between body weight and leptin concentration at term suggests that adipose tissue mass is a major determinant of leptin secretion in utero and participates actively in the adaptation of fetus to intrauterine hypoxia. Regulation of cord blood leptin level can be accepted as a complex process involving fetal gender and fetal anthropometric variables as well as cord blood cortisol, intrauterine growth and hypoxia.

We thank Dr. F. Rana Toktas and Ibrahim Bayram for their help in performing the leptin and cortisol assays.

References

1. 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;372:425-32.
2. Considine RV, Sinha MK, Heiman ML; Kriauciunas A, Stephens TW, Nyce MR, et al. Serum immunoreactive-leptin concentrations in normal-weight and obese humans. N Engl J Med 1996;334:292-5.
3. Halaas JL, Gajiwala KS,Maffei M, Cohen SL, Cahit BT, Rabinowitz D, et al. Weight-reducing effects of the plasma protein encoded by the obese gene. Science 1995;269:543-6.
4. Tamura T, Goldenberg RL, Johnston KE, Cliver SP. Serum leptin concentrations during pregnancy and their relationship to fetal growth. Obstet Gynecol 1998;91:389-95.
5. Lepercq J, Lahlou N, Timsit J, Girard J, Hauguel-de Mouzon S. Macrosomia revisited: Ponderal index and leptin delineate subtypes of fetal overgrowth. Am J Obstet Gynecol 1999;181:621-5.
6. Wiznitzer A, Furman B, Zuili I, Shany , Reece EA, Mazor M. Cord leptin level and fetal macrosomia. Obstet Gynecol 2000;96:707-13.
7. Geary M, Pringle PJ, Persaud M, Wilshin J, Hindmarsh PC, Rodeck CH; Brook CGD. Leptin concentrations in maternal serum and cord blood: relationship to maternal

anthropometry and fetal growth. Br J Obstet Gynaecol 1999;106:1054-60.

8. Masuzaki H, Ogawa Y, Sagawa N, Hosoda K, Matsumoto T, Mise H, et al. Nonadipose tissue production of leptin: leptin as a novel placenta-derived hormone in humans. Nat Med 1997;3:1029-33.
9. Laml T, Hartmann BW, Ruecklinger E, Preyer O, Soeregi G, Wagenbichler P. Maternal serum leptin concentrations do not correlate with cord leptin concentrations in normal pregnancy. J Soc Gynecol Invest 2001;8:43-7.
10. Ødegård RA, Vatten LJ, Nilsen ST, Salvesen KÅ, Austgulen R. Umbilical cord plasma leptin is increased in preeclampsia. Am J Obstet Gynecol 2002;186:427-32.
11. World Health Organisation. WHO Expert Committee on Diabetes Mellitus: second report. Geneva: World Health Organisation; 1980. Tech Rep Ser No.: 646.
12. Kubli FW, Hon EH, Khazin AF, Takemura H. Observations on heart rate on pH in human fetus during labor. Am J Obstet Gynecol 1969; 104: 1190-1206.
13. Clapp JF III, Kiess W. Cord blood leptin reflects fetal fat mass. J Soc Gynecol Invest 1998;5:300-3.
14. Koistinen HA, Koivisto VA, Andersson S, Karonen SL, Kontula K, Oksanen L, Teramo KA. Leptin concentration in cord blood correlates with intrauterine growth. J Clin Endocrinol Metab 1997;82:3328-30.20
15. Lepercq J, Guerre-Millo M, Andre J, Caüzac M, Hauguel-de Mouzon S. Leptin: a potential marker of placental insufficiency. Gynecol Obstet Invest 2003; 55: 151-5.
16. Schubring C, Kiess W, Englaro P, Rascher W, Dotsch J, Hanitsch S, Attanasio A, Blum WF. Levels of leptin in maternal serum, amniotic fluid, and arterial and venous cord blood: relation to neonatal and placental weight. J Clin Endocrinol Metab. 1997;82(5):1480-3.
17. Laml T, Preyer O, Schulz-Lobmeyr I, Ruecklinger E, Hartmann BW, Wagenbichler P. Umbilical venous leptin concentration and gender in newborn. J Soc Gynecol Invest 2001;8:94-7.
18. Lepercq J, Challier JC, Guerre-Millo M, Cauzac M, Vidal H, Hauguel-de Mouzon S. Prenatal leptin production: Evidence that fetal adipose tissue produces leptin. J Clin Endocrinol Metab 2001;86:2409-13.
19. Lea RG, Howe D, Hannah LT, Bonneau O, Hunter L, Hoggard N. Placental leptin in normal, diabetic and fetal growth-retarded pregnancies. Mol Hum Reprod 2000; 6:763-9.
20. Varvarigou A, Mantzoros CS, Beratis NG,. Cord blood leptin concentration in relation to intrauterine growth. Clin Endocrinol 1999;50:177-83.
21. Shekhawat PS, Garland JS, Shivpuri C, Mick GJ, Sasidharan P, Pelz CJ, et al. Neonatal cord blood leptin: Its relationship to birth weight, body mass index, maternal diabetes, and steroids. Pediatr Res 1998;43:338-43.
22. Cetin I, Morpurgo PS, Radaelli T, Taricco E, Cortellazzi D, Bellotti M, et al. Fetal plasma leptin concentrations: Relationship with different intrauterine growth patterns from 19 weeks to term. Pediatr Res 2000;48:646-51.
23. Takahashi Y, Yokoyama Y, Kawabata I, Iwasa S, Tamaya T. Leptin as an acute stress-related hormone in the fetoplacental circulation. Obstet Gynecol 2002;100:655-8.
24. Buchbinder A, Lang U, Baker RS, Khourny JC, Mershon J, Clarck KE. Leptin in the ovine fetus correlates with fetal and placental size. Am J Obstet Gynecol 2001;185:786-91.
25. Mise H, Sagawa N, Matsumoto T, Yura S, Nanno H, Itoh H, Mori T, Masuzaki H, Hosoda K, Ogawa Y, Nakao K. Augmented placental production of leptin in preeclampsia: possible involvement of placental hypoxia. J Clin Endocrinol Metab. 1998;83(9):3225-9.
26. Maffeis C, Moghetti P, Vettor R , Lombardi AM, Vecchini S, Tato L. Leptin concentrations in newborns' cord blood: Relationship to gender and growth-regulating hormones. Int J Obes 1999;23:943-7.
27. Catalano PM, Drago NM, Amini SB. Factors affecting fetal growth and body composition. Am J Obstet Gynecol 1995;172:1459-63.
28. Raff H, Bruder ED, Jankowski BM, Colman RJ. Effect of neonatal hypoxia on leptin, insulin, growth hormone and body composition in the rat. Horm Metab Res 2001; 33:151-5.
29. Vitoratos N, Chrystodoulacos E, Kouskouni E, Salamalekis E, Creatsas . Alterations of the maternal and fetal leptin concentrations in hypertensive disorders of pregnancy. Eur J Obstet Gynecol Rep Biol 2001;96:59-62.