Assessing leptin and soluble leptin receptor levels in full-term asymmetric small for gestational age and healthy neonates

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SUMMARY: Aydın Hİ, Demirkaya E, Karadeniz RS, Olgun A, Alpay F. Assessing leptin and soluble leptin receptor levels in full-term asymmetric small for gestational age and healthy neonates. Turk J Pediatr 2014; 56: 250-258.

The aim in this study was to determine the factors affecting leptin and soluble leptin receptor (sOB-R) levels in term small for gestational age (SGA) and appropriate for gestational age (AGA) newborns. The study group consisted of SGA (n=20) and AGA (n=20) newborns and their mothers. The leptin and sOB-R levels were tested using the ELISA method. The cord blood leptin concentrations were found significantly higher in the AGA group than in the SGA group (p=0.048). It was observed that cord blood leptin levels increased as body weight increased in the AGA group (r=0.681, p=0.001). The cord blood leptin levels were found higher in female infants than male infants (p=0.021). The plasma leptin levels were higher in the mothers of SGA neonates than those of AGA neonates (p=0.014). A positive correlation was detected between cord blood and amniotic fluid sOB-R concentrations in the AGA group (AGA: r=0.492, p=0.028). We conclude that the main determinants of leptin in SGA and AGA newborns are different. We can state that birth weight and gender are the main determinants of leptin levels in healthy neonates, but factors other than birth weight and gender may contribute to leptin levels in SGA newborns.

Key words: leptin, soluble leptin receptor, sOB-R, small for gestational age (SGA), intrauterine growth retardation (IUGR).

Intrauterine growth retardation (IUGR), due to abnormal maternal, fetal or placental factors, causes birth of small for gestational age (SGA) neonates. Evidences of an association of SGA with increased neonatal morbidity and mortality along with increased risk for cardiovascular diseases, obesity, insulin resistance, and metabolic syndrome in later life have been well documented in the literature[1,2]. The fetus exposed to IUGR develops many metabolic adaptations in response to reduced nutrient supply. Such metabolic adaptations are necessary for survival of the fetus and are achieved through enhancing the capacity of the fetus in taking up and using nutritional components[3]. One of these metabolic adaptations is associated with adipokines, especially leptin, which have important regulatory effects during the late gestational period when intrauterine growth is at its fastest rate[4]. It has been shown that leptin,

which is encoded by the obesity (ob) gene and synthesized mainly by the adipocytes, is also secreted in significant amounts by the placental trophoblastic cells during gestation. Leptin is also found in maternal, fetal, and umbilical circulation, and is increasingly elevated at the beginning of the second gestational trimester[5,6]. Leptin has been found to have significant effects on fetal growth in addition to its other effects in the regulation of adipose tissue and energy homeostasis through appetite control. It has also been noted that low leptin level in cord blood is a risk factor for SGA neonates[7]. Our study aimed to measure levels of leptin and soluble leptin receptor (sOB-R) in venous cord blood and amniotic fluid in AGA (appropriate for gestational age) and SGA term neonates. In addition, we also investigated the association of leptin and sOB-R with fetal growth.

Material and Methods

This study was planned through a cross-sectional design conducted on pregnant women and their neonates, seeking care and delivered, respectively, at Zübeyde Hanım Maternity and Child Care Hospital and Gülhane Military Medical Academy, between January 2005 and December 2009. The study was approved and supported by the institutional ethical committee. Written informed consents were obtained from the participants and for neonates from their respective parents. All the pregnant women included in the investigation were in term gestation with respect to the last menstrual period. The mothers were evaluated according to the inclusion criteria given in Table I, and the mothers meeting the criteria (n=395) were included in the study. Samples of venous blood were also obtained for assessing leptin level prior to delivery from all pregnant women during the collection of blood samples for other investigations. For checking the leptin level in neonates (n=176), samples were taken from umbilical cord blood by placing a second clamp on the umbilical cord following cesarean section. The blood samples were centrifuged at 5000 rpm for 10 minutes (min), and serum samples were stored at -20°C until analysis. The samples of amniotic fluid were also collected using an injector before the amniotic membrane was opened, and the samples were centrifuged at 5000 rpm for 10 min and stored at -20°C before determining leptin and sOB-R levels. Leptin and sOB-R levels were measured using the enzyme-linked immunosorbent assay (ELISA) method with commercially available test kits (BioVendor Laboratory Medicine, Modrice, Czech Republic).

The neonates (n=176) whose blood samples were collected for leptin and sOB-R tests were evaluated according to the inclusion criteria given in Table I, and the 40 infants who met the inclusion criteria were included in the study. The gestational ages of all neonates were assessed using the Dubowitz scoring method. Neonates who were evaluated as term neonates according to the last menstrual period and the Dubowitz scoring method were included in the study. Their birth weights according to gestational age were evaluated with respect to growth curves. Liveborn infants with birth weight less than the 3rd percentile according

to gestational age were considered as SGA and infants with birth weight between 10-90 percentiles according to gestational age as AGA (Table II). According to birth weight, an equal number of infants met the criterion to be considered in AGA (n=20) and SGA (n=20) groups. Ponderal index (PI) was used to determine the growth patterns of the term SGA infants. PI was calculated using the standard formula [weight (grams) / height^3 (cm) x 100], and the infants with PI <2.32 were assessed as asymmetric IUGR[8].

Data were analyzed using the Statistical Package for the Social Sciences version 20 (SPSS Inc., Chicago, IL). Student t test and Mann-Whitney U test were used for comparing the quantitative data in addition to other definitive statistical methods (mean, standard deviation). The correlations between the groups were evaluated using Pearson correlation. The results were evaluated within 95% confidence interval (CI), and p<0.05 was accepted as the level of significance.

Results

No difference was found between the AGA and SGA groups with respect to gestational age (p=0.308). Both groups included 10 male and 10 female infants. No statistical difference was detected between the male and female infants with respect to maternal age, gestational age, neonatal birth weight, and levels of maternal leptin, soluble leptin, leptin, and sOB-R in the amniotic fluid. PI was higher in the AGA group when compared to the SGA group (p=0.001) (Table II).

We found that leptin levels in cord blood were higher in female compared to the male infants (p=0.021) (Table III). Moreover, cord blood leptin concentrations were found significantly higher in the AGA group when compared to the SGA group (p=0.048). However, no correlation was observed between cord blood leptin concentration and other factors such as maternal age, gestational age and neonatal birth weight. While evaluating both groups (AGA and SGA) concurrently, no statistical correlation was found between cord blood leptin concentrations and neonatal birth weight. However, when the AGA and SGA groups were evaluated separately, although there was no correlation between body weight and cord blood leptin levels in the SGA group (r=0.406,

Table I. Inclusion Criteria for Mothers and their Neonates

Inclusion Criteria for Mothers
Single gestation
Nonsmoker
No history of alcohol or drug abuse
No finding of preeclampsia
No diabetes mellitus/gestational diabetes mellitus
No medical history of using chronic medication during gestation
Delivery with cesarean section
Inclusion Criteria for AGA
Gestational age more than 37 weeks and earlier than 40 weeks (according to last menstrual period and Dubowitz Scoring System)
Birth weight between 10th and 90th percentiles
Birth without meconium
No major anomaly
No finding of birth asphyxia
First and fifth minute APGAR scores above 7
Inclusion Criteria for SGA
Gestational age more than 37 weeks and earlier than 40 weeks (according to last menstrual period and Dubowitz Scoring System)
Birth weight below 3rd percentile
Ponderal index below 2.32
Birth without meconium
No major anomaly
No finding of birth asphyxia
First and fifth minute APGAR scores above 7

p=0.076), the AGA group showed elevated cord blood leptin levels as body weight increased (r=0.681, p=0.001) (Fig. 2). A statistically significant difference in maternal plasma leptin concentrations was found between the AGA and SGA groups (p=0.001) (Table II), and unlike with cord blood leptin, here the SGA group showed a higher value. Similar to cord blood leptin concentration, no correlation was found between maternal leptin levels and other factors such as maternal age, neonatal birth weight, gestational age, and gender (neonate). Maternal plasma leptin levels showed a positive correlation with maternal body mass index (BMI) in the AGA group (AGA: r=0.772, p=0.001); however, no such correlation was detected in the SGA group (SGA: r=0.165, p=0.487) (Fig. 1). While analyzing amniotic fluid, leptin concentrations were found significantly higher in the AGA group than the SGA group (p=0.012). We did not find any correlation between amniotic fluid leptin concentrations and other factors such as maternal age, gestational age, gender (neonate), neonatal birth weight, maternal leptin level, and infant leptin level in either group.

The sOB-R concentrations, both in cord blood and amniotic fluid, were found significantly lower in the AGA group compared to the SGA group (p=0.001) (Table II). We did not find any correlation between the sOB-R concentrations in the cord blood and amniotic fluid with other factors such as maternal age, gestational age, gender (neonate), neonatal birth weight, and levels of maternal leptin and infant leptin in either group (AGA and SGA); however, a positive correlation was found between sOB-R concentrations in the cord blood and amniotic fluid between the two groups (AGA: r=0.492, p=0.028; SGA: r=0.785, p=0.001) (Fig. 4).

We evaluated the effect of PI on leptin and sOB-R levels in the AGA and SGA groups. We found a positive correlation between PI and cord blood leptin levels (r=0.476, p=0.002) (Fig. 3). PI was higher in the AGA group when compared to the SGA group (p=0.001). Cord blood leptin levels elevated as PI increased in the AGA group (r=0.546, p=0.013), but no correlation was found between PI and cord blood leptin levels in the SGA group (r=0.390, p=0.086) (Fig. 3). There was no correlation between PI and sOB-R in cord blood or

Table II. Comparison between AGA and SGA Groups

	AGA			SGA			p
	Mean	SD	Interval	Mean	SD	Interval	
Maternal Age (years)	29.55	2.45	25-35	29.40	2.45	25-34	0.348
Gestational Age (weeks)	38.2	0.7	37-40	37.9	0.9	37-40	0.308
Birth Weight (grams)	3072	162	2750-3450	2129	173	1840-2400	0.001
Ponderal Index	2.59	0.17	2.41-3.03	1.99	0.16	1.74-2.30	0.001
Leptin* (CB)	19.96	10.01	6.17-42.73	13.75	9.18	2.69-34.44	0.048
Leptin* (AF)	13.21	4.94	3.43-21.48	9.63	3.43	1.48-15.02	0.012
sOB-R* (CB)	5.28	1.81	1.91-8.58	8.41	3.03	4.66-16.50	0.001
sOB-R* (AF)	3.17	3.18	1.25-16.25	8.06	2.88	4.01-14.26	0.001
Leptin (CB)/weight**	6.40	2.94	2.00-12.38	6.35	4.11	1.46-15.65	0.941
Leptin (AF)/weight**	4.28	1.55	1.18-7.04	4.51	1.52	0.69-6.99	0.642
sOB-R (CB)/ weight **	1.73	0.61	0.58-2.95	3.95	1.40	2.05-8.05	0.001
sOB-R (AF)/ weight **	1.04	1.05	0.41-5.33	3.70	1.40	1.97-6.25	0.001
Leptin (mother)	33.78	22.81	1.52-77.16	52.82	23.95	17.44-85.18	0.014
BMI (mother) (kg/m^2)	23.20	2.32	19.45-27.89	23.68	1.88	20.10-26.62	0.481

AF: Amniotic fluid. BMI: Body mass index. CB: Cord blood. sOB-R: Soluble leptin receptor. **ng/ml. **Values were corrected for body weight (kg). SD: Standard deviation. Values were shown as mean ± SD (Student t test).

Table III. Comparison between Male and Female Patients

	Female			Male			p
	Mean	SD	Interval	Mean	SD	Interval	
Maternal Age (years)	30.50	2.13	27-35	28.45	2.21	25-32	0.087
Gestational Age (weeks)	38.1	0.6	37-39	37.9	0.9	37-40	0.575
Birth Weight (grams)	2613	479	1910-3300	2588	542	1840-3450	0.878
Ponderal Index	2.28	0.31	1.91-2.94	2.30	0.36	1.74-3.03	0.829
Leptin* (CB)	20.45	9.56	6.73-38.84	13.26	9.28	2.69-42.73	0.021
Leptin* (AF)	12.51	4.41	4.87-21.48	10.32	4.57	1.48-16.42	0.131
sOB-R* (CB)	7.03	3.27	3.41-16.50	6.67	2.61	1.91-11.91	0.741
sOB-R* (AF)	5.18	3.32	1.58-13.75	5.90	3.98	1.25-16.25	0.542
Leptin (mother)	45.73	23.62	17.44-85.18	40.87	26.72	1.52-77.16	0.546
Leptin (CB)/weight**	7.85	3.60	2.79+/-15.65	4.90	2.83	1.46-12.38	0.007

AF: Amniotic fluid. BMI: Body mass index. CB: Cord blood. sOB-R: Soluble leptin receptor. **ng/ml. **Values were corrected for body weight (kg). SD: Standard deviation. Values were shown as mean ± SD (Student t test).

amniotic fluid.

In order to evaluate the effect of neonatal birth weight on leptin and sOB-R levels, the difference between the AGA and SGA groups was reevaluated by correcting leptin and sOB-R levels in cord blood and amniotic fluid using formulas of neonatal birth weight (leptin (ng/ml)/body weight (kg) and sOB-R (ng/ml)/body weight (kg)). We found that the differences between leptin levels in cord blood and amniotic fluid in the AGA and SGA groups disappeared
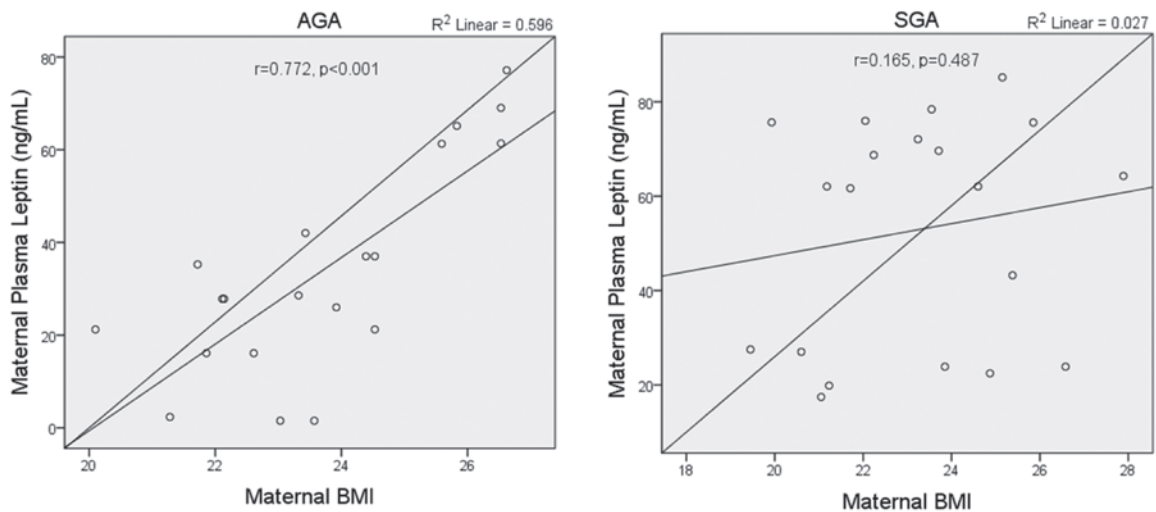
Fig. 1. The correlation between maternal plasma leptin level and maternal BMI in the AGA and SGA groups.
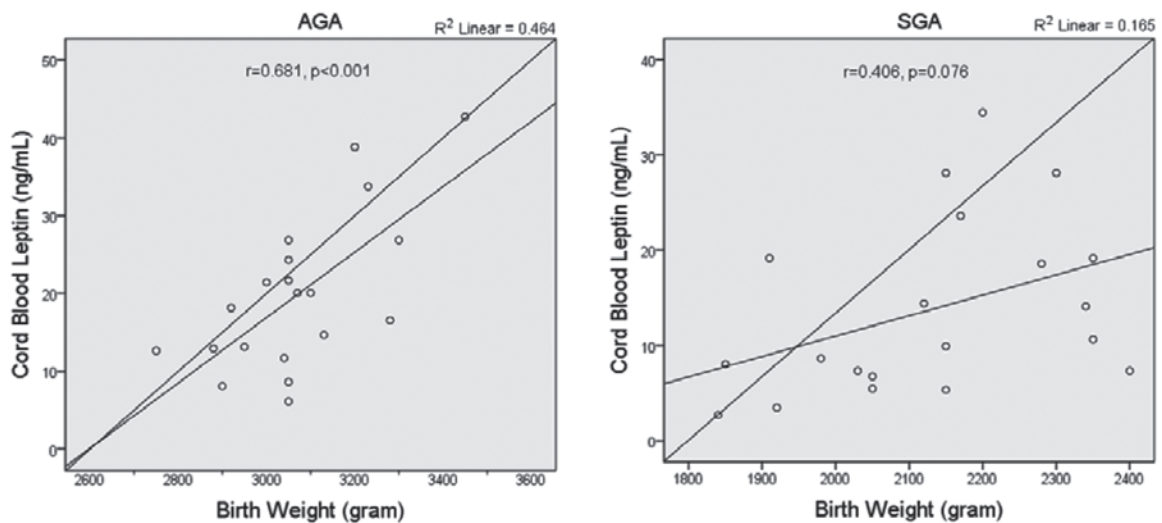

Fig. 2. The correlation between cord blood leptin level and birth weight in the AGA and SGA groups.

after correction of leptin levels using the formula of neonatal birth weight (Table II). The statistical analysis also revealed that the differences in sOB-R levels in cord blood and amniotic fluid between the AGA and SGA groups continued even after the correction of leptin levels using the formula of neonatal birth weight (Table II). Since female infants were detected to have a higher body weight and cord blood leptin concentration compared to male infants, we investigated whether this difference in cord blood leptin concentration in male and female infants originated from the difference in body weights. We found that the higher

leptin levels in female infants persisted even after cord blood leptin levels were corrected using the formula for neonatal birth weight (leptin (ng/ml)/body weight (kg)) (Table III).

Discussion

Leptin detected in cord blood is mostly sourced from fetal adipose tissue and is synthesized in the fetus beginning from the 18th week, reaching remarkable levels at the beginning of the 34th week when fetal adipose tissue is increased. Lower leptin levels in the cord blood of SGA neonates suggest the probable association of metabolic adaptation with leptin due to IUGR[9,10]. Similar to the previous reports,
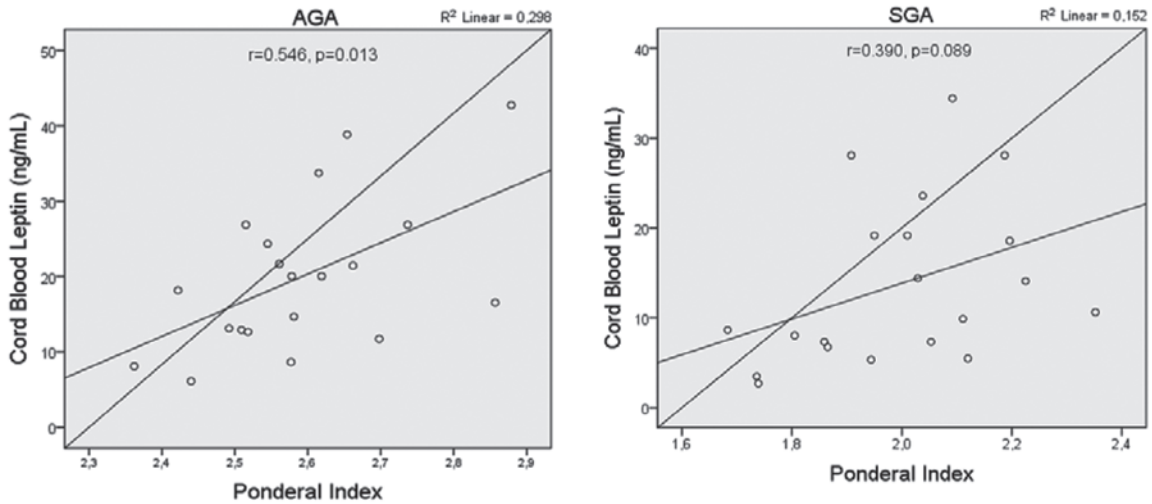
Fig. 3. The correlation between cord blood leptin level ponderal index in the AGA and SGA groups.
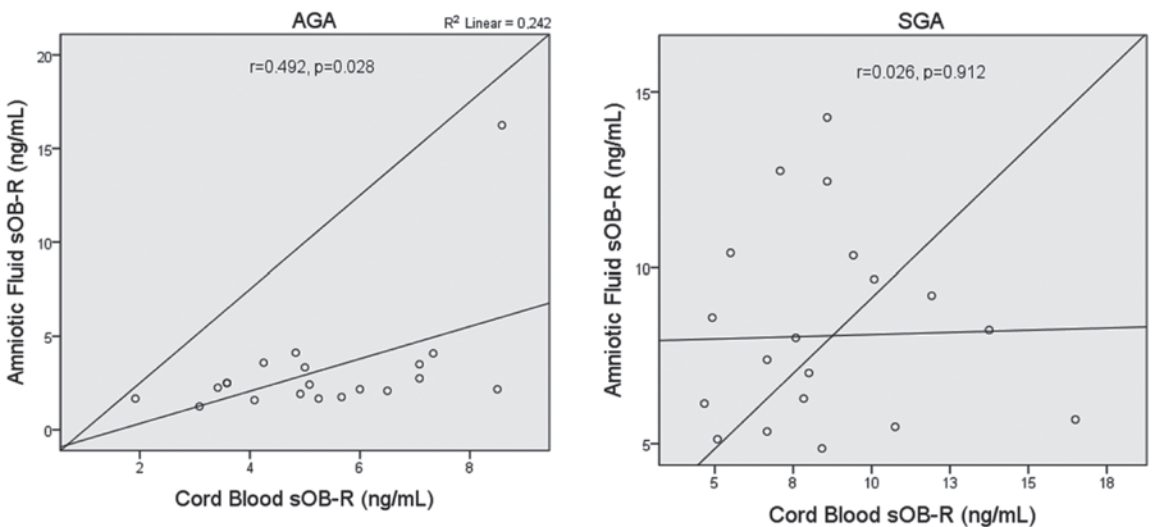

Fig. 4. The correlation between sOB-R levels in amniotic fluid and cord blood in the AGA and SGA groups.

we found a lower level of cord blood leptin in the SGA group compared to the AGA group. We also found a positive correlation between cord blood leptin levels and birth weight in both SGA and AGA neonates, with the AGA group showing a remarkable correlation. This stronger correlation in the AGA group suggests body weight as the main determinative factor for cord blood leptin levels.

We used PI to select asymmetric SGA newborns in our study. PI is widely used in differentiating between symmetric and asymmetric SGAs. Only asymmetric SGA neonates with PI below 2.32 were included in our study. Low PI is especially associated with low adipose tissue due to

impaired fetal nutrition in the period when leptin synthesis is remarkably increased[8,10]. We found a significant association between leptin and PI in the AGA group, but not in the SGA group. Our results are in accordance with the finding of an association between cord blood leptin and indices of fetal growth, including birth weight and PI, in the AGA group[11-13]. The weaker correlation of cord blood leptin with birth weight and PI in the SGA neonates indicates involvement of other factors such as hypoxia, which has been shown to induce leptin synthesis, might be contributing in addition to body weight[14]. In our study, in the selection of SGA neonates, clinical criteria

(i.e., no meconium staining, no findings of asphyxia after birth or apnea, and normal APGAR scores) were accepted as nonexistence of remarkable intrauterine hypoxia[15]. Moreover, the weaker correlation may also point towards an increased ratio of leptin sourced from the other fetal tissues, because in the SGA group, adipose mass is proportionally lower than body mass, and the presence of hypoxia is below the clinically symptomatic level.

Grisaru-Granovsky et al.[16] suggested the placental factor might have some contribution to the lower body weight and cord blood leptin of neonates. Although a positive correlation has been shown between cord blood leptin and placenta size, 98.4% of leptin synthesized in the placenta enters maternal circulation, whereas leptin detected in the cord blood is mostly sourced from fetal adipose tissue and other fetal tissues[17,18]. We found higher levels of plasma leptin in mothers delivering SGA neonates compared to those delivering neonates in the AGA group. If cord blood leptin levels of the neonates in the SGA group had a placental source, then the cord blood leptin level would have been expected to be higher in the SGA group than the AGA group. However, we obtained the opposite result (SGA < AGA); therefore, our findings suggested that cord blood leptin in our patients delivering SGA neonates had a fetal source, supporting the findings of Linnemann et al.[18] Leptin levels elevate two-fold higher in pregnant women compared to non-pregnant ones[5], and further higher elevations can be seen in case of pregnancy complications such as gestational diabetes mellitus, preeclampsia, and IUGR[19,20]. Serum leptin levels in women are generally associated with adipose tissue mass and show a correlation with BMI[5,21]. However, the elevation of plasma leptin levels during pregnancy is too high to be explained with adipose tissue mass only[22]. Such alterations in leptin level occur for mobilization of nutrient sources required for fetal growth such as maternal adipose tissue[9]. We also found a positive correlation between plasma leptin levels and BMI (r=0.772, p=0.001) in mothers of AGA neonates; however, no such correlation was found in mothers of SGA neonates (r=0.165, p>0.05). It has also been reported that neonatal body weight has a determinative effect on maternal plasma leptin levels during pregnancy[20].

We found leptin levels of the mothers of SGA infants to be higher than those of the AGA group (p=0.014). Grosfeld et al.[23] found higher plasma leptin levels in mothers with preeclampsia and hypoxia developed due to placental dysfunction, which increases placental leptin expression through hypoxia inducible factor (HIF)-1 in such patients. Mothers participating in our study had no clinical or laboratory findings of preeclampsia or gestational diabetes mellitus. Indisputably, eclamptic mothers, giving birth, irrespective of AGA or SGA neonates, have increased plasma leptin levels[20,24,25]. Recently, some studies have revealed increased placental leptin mRNA expression in mothers of SGA neonates having higher plasma leptin levels than the mothers of AGA neonates[19,20]. Therefore, maternal plasma leptin levels can be increased due to increased placental leptin expression in mothers of SGA neonates without a clinical or laboratory finding of preeclampsia, and placental leptin expression does not affect plasma leptin levels in the fetus. In addition, similar to cord blood leptin levels, our study also showed lower amniotic fluid leptin levels in the SGA group compared to the AGA group.

Next, we investigated the effect of gender on leptin synthesis and found that female neonates had higher cord blood leptin levels than male neonates (p=0.021). Birth weights of female infants were also higher than of male infants, although the difference was not statistically significant. A statistically significant difference was established between genders when leptin levels were corrected for body weight (p=0.007). Our findings are in agreement with the previous studies both in terms of gender contribution in leptin synthesis and of female infants having higher leptin levels (11,26-28). However, gender-dependent body adipose mass and reproductive hormones such as testosterone and estradiol are not involved; rather, a genetic factor might play a role. Advanced investigations are required to establish a mechanism of gender factor involvement[11,27,28].

We also investigated the relation of sOB-R levels in cord blood and amniotic fluid with neonatal body weight and leptin levels. We found that cord blood and amniotic fluid sOB-R concentrations of SGA neonates were higher

than of AGA neonates irrespective of body weight (p=0.001). This finding is consistent with previous studies that have demonstrated the association of energy deficiency with an increased rate of apoptosis and elevated levels of sOB-R. High levels of sOB-R in SGA newborns are also thought to be compensatory to energy deprivation in the fetus[29,30]. We also showed a positive correlation between cord blood and amniotic fluid sOB-R levels in AGA newborns. It has been shown that sOB-R levels in umbilical venous or arterial blood are similar, and no correlation was found between mother and infant with respect to cord blood sOb-R levels in previous studies. Existence of a correlation between cord blood and amniotic fluid in our study reconfirmed the finding that cord blood sOb-R may have a fetal, not placental, source[30].

Our study has some limitations. Since the placenta was not evaluated, we could not assess the contribution of a placental factor in IUGR. In addition, maternal weight gain during pregnancy, postpartum adiposity and maternal nutritional history were not evaluated in this study.

In conclusion, our study showed that the main determinants of leptin in SGA and AGA newborns are different. Birth weight and gender are the main determinants of leptin levels in healthy neonates. The underlying reasons for the gender-dependent difference in leptin levels is not yet known and requires further investigations. In SGA newborns, factors other than birth weight and gender might contribute to leptin levels.

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