Serum leptin levels of premature and full-term newborns in early infancy: metabolic catch-up of premature babies

Demet Toprak[1], Ayşe Sevim Gökalp[1], Şükrü Hatun[1], Emine Zengin[1]
Ayşe Engin Arısoy[1], Zeki Yumuk[2]

Departments of [1]Pediatrics, and [2]Microbiology and Clinical Microbiology, Kocaeli University Faculty of Medicine, Kocaeli, Turkey

SUMMARY: Toprak D, Gökalp AS, Hatun S, Zengin E, Arısoy AE, Yumuk Z. Serum leptin levels of premature and full-term newborns in early infancy: metabolic catch-up of premature babies. Turk J Pediatr 2004; 46: 232-238.

Leptin is secreted from the edipose tissue and has an important role in the regulation of energy metabolism. This study aimed to compare serum leptin levels of preterm and full-term infants during the first three months of their life and to define the roles of sex, weight, thickness of subcutaneous adipose tissue, gestational age and maternal leptin in the determination of serum leptin levels.

Forty-four full-term and 32 preterm infants were included in the study. Weight, thickness of subcutaneous adipose tissue, serum glucose, cortisol, insulin and leptin levels were compared between preterm and full-term infants at 7th, 30th and 90th days. ELISA method was used in determining serum leptin levels.

Weight, thickness of subcutaneous adipose tissue and serum leptin levels were significantly increased in full-term infants compared to preterm infants at days 7 and 30. At 90th day weight and thickness of subcutaneous adipose tissue were significantly increased in full-term infants, but the difference in serum leptin levels did not reach statistical significance (p=0.56). Weight was the most important factor predicting serum leptin levels at the 7th day. On the other hand, the thickness of subcutaneous adipose tissue was the most important determinant at days 30 and 90. Maternal serum leptin level was a determinant of serum leptin level at day 7. Sex was a determinant of serum leptin level of the infant at days 7 and 30. The differences in weight gain, increase in thickness of subcutaneous adipose tissue and increase of serum leptin levels were not significant between groups. But the increase in serum leptin levels was correlated in both preterm and full-term infants with weight gain and increase in thickness of subcutaneous adipose tissue.

At three months of age, in the catch-up growth period, preterm infants reach serum leptin levels near those of full-term infants. The thickness of subcutaneous adipose tissue has a role in the determination of serum leptin levels after 30 days of life.

***Key words:** preterm infant, full-term infant, leptin, thickness of subcutaneous adipose tissue.*

Leptin is a 16 kD adipocyte-specific protein, which regulates body weight through a negative feedback mechanism between adipose tissue and the hypothalamic centers of the brain[1]. Previous studies have also demonstrated that leptin exists at high concentrations in cord blood and its concentration correlates with gestational age, birth weight and several maternal factors. Leptin was found to be detectable in human fetal cord blood as early as 18 weeks of gestation and its serum concentration was independent of maternal serum concentration of leptin[2,3].

The accumulation of the body fat mass was reported as a major determinant of serum leptin levels in the fetus[4]. Therefore, small for gestational age and premature newborns have reduced serum leptin concentrations[5,6]. The mode of delivery and diet, gestational age, serum levels of insulin, glucocorticoids and androgens, resting metabolic rate and gender were among other factors influencing the circulating serum leptin level in the newborn[7].

Leptin synthesis is stimulated by the hormones that regulate body energy homeostasis. Serum leptin levels of the newborn have been shown to decrease progressively at 3rd to 7th days after birth. The rapid decrease of leptin levels after birth could be mediated by hormonal changes after birth. This was thought to be a physiologic advantage for newborns, especially for premature and small for gestational age babies, because energy supplies provided during intrauterine life were preserved in this way[4].

The aim of this study was to determine factors affecting serum leptin levels of the newborn, and to compare premature and term babies with regard to the increase in body weight and subcutaneous adipose tissue thickness on serum leptin levels in the first 90 days after birth.

Material and Methods

Study design and subjects

Forty-four full-term and 32 premature healthy newborn infants and their mothers were included in this cross-sectional study. Corticosteroid use, neonatal infection, congenital anomaly and chronic disease were exlusion criteria for this study. The gestational age was determined according to the New Ballard's scoring system and the babies were classified as small, appropriate and large for gestational age according to the Lubchenko curves. Head circumference, height, weight and ultrasonographic adipose tissue thickness were measured in all participants.

This study was approved by the Ethical Committee of Kocaeli University and was conducted according to the principles of the declaration of Helsinki. Informed consent was obtained from all parents before their participation in the study.

Blood sampling

All venous blood samples from the babies and their mothers were drawn by venipuncture. Blood samples of the babies were taken at the 7th, 30th and 90th days after two hours of fasting, and their mothers' were sampled at the 7th postnatal day after 12 hours of fasting. After serum glucose, cortisol and insulin levels were measured, the rest of the blood sample was centrifuged immediately and stored at –20°C until serum leptin assay.

Laboratory methods

All serum samples were studied by the enzyme-linked immunosorbent assay (ELISA) method using a commercial kit provided by Diagnostics Systems Laboratories Inc. (DSL-10-23100 human leptin enzyme-linked immunosorbent kits). Hemolyzed and lipemic serums were not used. The sensitivity or minimum detection limit of the assay, as calculated by interpolation of the mean plus two standard deviations of 12 replicates of the 0 ng/ml Human Leptin Standard, was 0.05 ng/ml. Serum insulin and cortisol levels were determined by a chemiluminescent method on an Immulite automated analyzer (Immulite 2000, Diagnostic Products Corporation, Los Angeles, USA). Serum glucose was measured with the glucose oxidase method by an auto-analyzer (Tecnicon-Bayer DAX-48).

Measurement of subcutaneous adipose tissue thickness

The thickness of subcutaneous adipose tissue at the proximal third of the left humerus was measured by a 12 MHz linear ultrasound transducer (Toshiba Power Vision 8000). The ultrasonographic measurement of subcutaneous adipose tissue thickness has been validated previously using computer tomography as a reference standard[8].

Statistical analysis

All calculations were conducted using SPSS computer software. Data were expressed as mean ± SD. The comparison between clinical characteristics, subcutaneous adipose tissue thickness and serum leptin levels of premature and full-term babies was done using t-test and χ^2-test, where appropriate. Within group comparisons were done using analysis of variance (ANOVA) at days 7, 30 and 90. To define the determinants of serum leptin levels, stepwise multiple regression analysis was performed. A two-tailed p value less than 0.05 was considered significant.

Results

Demographic characteristics of 76 newborn babies are presented in Table I. Thirty-two (42%) newborns were premature and 44 (58%) were full-term babies. Their mean gestational age was 36.9±3.2 weeks and they were mostly (91%) appropriate for gestational age (AGA) newborns. Their birth weight ranged from 960 to 5000.

Table I. Demographic Characteristics of Newborn Babies

	n: 76
Gender (Female/Male)	38/38
Premature	32 (42%)
Full-term	44 (58%)
SGA	4 (5%)
AGA	69 (91%)
LGA	3 (4%)
Gestational age (wk)	36.9±3.2
Mode of delivery (NSD/CS)	49/27
Birth weight (g)	2858±935
(range)	960-5000
Maternal age (yr)	28±6

SGA: Small for gestational age; AGA: Appropriate for gestational age; LGA: Large for gestational age; NSD: Normal spontaneous delivery; CS: Cesarean section.

Premature and full-term babies were not different with regard to their gender and maternal age (p>0.05, Table II). Maternal leptin levels of the premature babies (18.6±15.5 ng/ml) and full-term babies (14.0±10.2 ng/ml) were not statistically different (p: 0.12). Premature babies were younger (33.8±2.2 weeks vs. 39.2±1.0 weeks, p<0.001) and had lower Apgar scores (7±2 vs. 9±1) compared to full-term babies. Premature babies had lower birth weight (2037±770 g), birth height (43.3±5.0 cm) and head circumference (30.6±3.0 cm) than term babies (3455±482 g, 51.0±2.2 cm, 35.0±1.9 cm, respectively, p<0.001 for all).

Body weight of the premature infants significantly increased from the 7th day (2001±794 g) to 90th day (4163±1212 g) after birth (p<0.001, Table III). Similarly, subcutaneous adipose tissue significantly increased in thickness from 1.74±0.59 mm at the 7th day to 2.62±0.98 mm at the 90th day (p<0.001). Concordant with this data, serum leptin levels were 0.88±0.97 ng/ml at 7th day and increased to 3.19±3.14 ng/ml at 30th day and to 4.81±3.76 ng/ml at 90th day (p<0.001, Fig. 1).

Table II. Demographic and Maternal Characteristics of Premature and Full-term Babies

	Premature (n: 32)	Full-term (n: 44)	p
Gender F/M	14/18	24/20	0.36
Maternal age (yr)	27±6	28±6	0.52
Gestational age (wk)	33.8±2.2	39.2±1.0	<0.001
Apgar score	7±2	9±1	<0.001
Birth weight (g)	2037±770	3455±482	<0.001
Birth height (cm)	43.3±5.0	51.0±2.2	<0.001
Head circumference (cm)	30.6±3.0	35.0±1.9	<0.001
Maternal leptin (ng/ml)	18.6±15.5	14.0±10.2	0.12

Table III. Body Weight, Subcutaneous Adipose Tissue Thickness and Serum Leptin Levels at 7, 30 and 90 Days After Birth in Premature and Full-term Babies

	7th day	30th day	90th day	p
Premature (n: 32)				
Body weight (g)	2001±794	2784±1211	4163±1212	<0.001
range	(1715-2288)	(2347-3220)	(4176-5050)	
Sc adipose tissue thickness (mm)	1.74±0.59	2.10±0.83	2.62±0.98	<0.001
range	(1.52-1.95)	(1.80-2.40)	(2.27-2.97)	
Serum leptin levels (ng/ml)	0.88±0.97	3.19±3.14	4.81±3.76	<0.001
range	(0.53-1.23)	(2.07-4.33)	(3.45-6.16)	
Full-term (n: 44)				
Body weight (g)	3632±514	4733±664	6319±785	<0.001
range	(3475-3788)	(4531-4934)	(6081-6558)	
Sc adipose tissue thickness (mm)	2.35±0.57	2.80±0.47	3.21±0.58	<0.001
range	(2.18-2.53)	(2.66-2.94)	(3.04-3.39)	
Serum leptin levels (ng/ml)	1.66±1.0	5.23±3.68	5.25±2.88	<0.001
range	(1.36-1.97)	(4.11-6.35)	(4.37-6.13)	

Sc: Subutaneous.

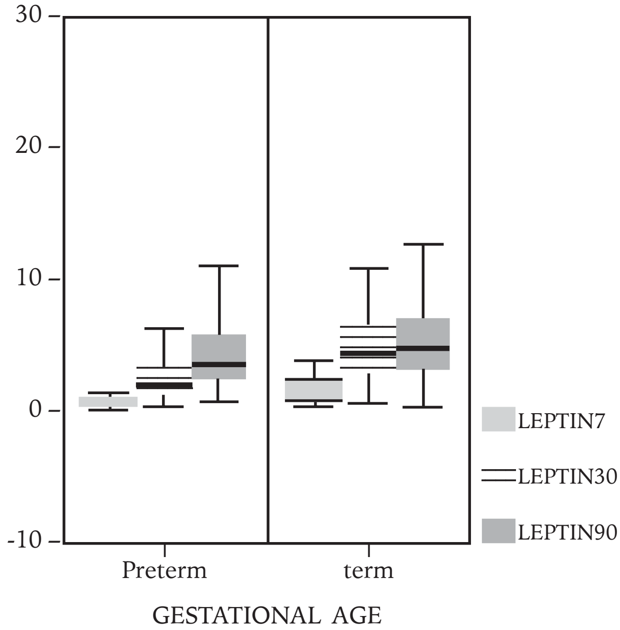

Fig. 1. Serum leptin levels of premature and term babies in the first ninety days after birth.

Relationship between body weight, subcutaneous adipose tissue thickness and serum leptin levels also followed a similar pattern in the full-term infants (Table III). Body weight at 7th day (3632±514 g) increased to 6319±785 g at 90th day in the full-term babies (p<0.001). Subcutaneous adipose tissue significantly increased from 2.35±0.57 mm at 7th day to 3.21±0.58 mm at 90th day (p<0.001). Meanwhile, serum leptin level of 1.66±1.0 ng/ml at 7th day increased to 5.25±2.88 ng/ml at 90th day (p<0.001, Fig. 1). Seventh, 30th and 90th day measurements of body weight, subcutaneous adipose tissue thickness and serum leptin levels were significantly higher in the full-term babies compared to premature babies (p<0.001 for all, Table III).

From the 7th to 30th day after birth, increase in body weight was significantly more pronounced in the full-term infants (1110±429 g) than in premature babies (721±441, p<0.001) (Table IV). Increase in subcutaneous adipose tissue thickness (0.45±0.65 mm vs. 0.36±0.79 mm, p: 0.61) and serum leptin levels (3.52±3.17 ng/ml vs. 2.30±2.87 ng/ml, p:0.09) did not reach statistical significance in the full-term and premature babies. Increases in body weight, subcutaneous adipose tissue thickness and serum leptin levels between the 30th and 90th day were not significantly different between premature and full-term babies (p<0.05, Table IV).

Table IV. Increase in Body Weight, Subcutaneous Adipose Tissue Thickness and Serum Leptin Level Between 7 to 30 Days and 30 to 90 Days in Premature and Full-term Babies

	Premature (n: 32)	Full-term (n: 44)	p
Increase between 7 to 30 days			
Body weight (g)	721±441	1110±429	<0.001
Sc adipose tissue thickness (mm)	0.36±0.79	0.45±0.65	0.61
Serum leptin levels (ng/ml)	2.30±2.87	3.52±3.17	0.09
Increase between 30 to 90 days			
Body weight (g)	1826±661	1586±611	0.11
Sc adipose tissue thickness (mm)	0.52±0.76	0.40±0.75	0.50
Serum leptin levels (ng/ml)	1.61±3.78	0.02±4.38	0.10

Sc: Subcutaneous.

Predictors of serum leptin concentrations

To define the independent predictors of serum leptin concentrations at 7th, 30th and 90th days, we modeled a multivariate analysis (Table V).

Table V. Independent Predictors of Serum Leptin Levels at 7th, 30th and 90th Days After Birth

Days	Predictors	Coefficients	t-test	P
7th day	Body weight	$9.2x10^{-4}$	5.03	<0.001
	Male gender	-0.687	-3.66	<0.001
	Sc adipose tissue	0.662	4.04	<0.001
	Maternal leptin	$1.74x10^{-2}$	2.37	0.02
	Gestational age	-0.137	-2.26	0.03
30th day	Male gender	-2.726	-4.17	<0.001
	Sc adipose tissue	1.444	2.53	0.01
	Body weight	$6.68x10^{-4}$	2.16	0.03
90th day	Sc adipose tissue	1.671	4.0	<0.001
	Body weight	$6.71x10^{-4}$	2.38	0.02

Gender was dummy coded as 0 for female, 1 for male. Sc: Subcutaneous.

Body weight and subcutaneous adipose tissue thickness were predictors of serum leptin levels at 7th, 30th and 90th days. Male gender was a negative predictor of serum leptin levels at 7th and 30th days, but did not influence serum leptin levels at 90th day. Maternal leptin level predicted serum leptin levels positively, but gestational age inversely determined leptin levels at 7th day.

Regression formula at 7th day = [3.745 +(9.2x10^{-4} x weight) + (0.662 x subcutaneous adipose tissue thickness) – (0.687 x [0 for female, 1 for male]) + (1.74x10^{-2} x maternal serum leptin level) – (0.137x gestational age)], R^2=0,46, p<0.001. Regression formula at 30th day = [-0.493 + (1.444 x subcutaneous adipose tissue thickness) – (2.726 x [0 for female, 1 for male]) + (6.68x10^{-4} x weight)], R^2=0.40, p=0.013. Regression formula at 90th day = [0.11+(1.671 x subcutaneous adipose tissue thickness)], R^2=0.18, p<0.001 and [1.304 + (6.71x10^{-4} x weight)], R^2=0.07, p=0.02.

There was no statistically significant difference in serum glucose, cortisol and insulin levels between premature and term babies (p>0.05). There was also no correlation between serum leptin levels and glucose, cortisol and insulin levels at days 7, 30 and 90 (Table VI).

Table VI. Correlation of Serum Leptin Levels and Glucose, Cortisol and Insulin Levels of Newborn Babies at 7, 30 and 90 Days

	Serum leptin levels	p
7th day		
glucose	r: -0.07	0.53
cortisol	r: -0.15	0.35
insulin	r: 0.27	0.10
30th day		
glucose	r: 0.07	0.57
cortisol	r: -0.19	0.32
insulin	r: 0.28	0.14
90th day		
glucose	r: 0.13	0.27
cortisol	r: -0.001	0.99
insulin	r: -0.06	0.82

r: correlation coefficient.

Discussion

This is the first prospective study that compares the serum leptin levels of premature and full-term infants throughout their first three months after birth. The serum leptin levels of premature and term infants increased progressively until the 90th day. Serum leptin concentrations of full-term babies were higher than prematures at 7th and 30th days, but this difference was not significant at 90th day. This data suggests that preterm infants increase their energy reserves and become adapted to extrauterine life like full-term infants just at the 90th day of life.

According to our results, the variability of serum leptin levels was correlated with the increase in body weight and subcutaneous adipose tissue thickness. This suggests that the weight gain and augmentation of adipose tissue have an important role as the infant grows and becomes adapted to extrauterine life.

Similar to previous studies, we considered that serum leptin at the 7th day was greatly associated with birth weight and gestational age. Ng et al.[7] have shown that the increase in serum leptin is most significant at 34 weeks of gestation. This increase corresponds mostly with the accumulation of fetal adipose tissue in the third trimester. A similar pattern of increase at around 32-34 weeks of gestation was observed by Matsuda et al.[9]. Furthermore, Ng et al.[7] also reported that there was an association between serum leptin and cortisol, or insulin and insulin: glucose ratio. In our study, there was no statistically significant correlation between serum leptin levels and serum glucose, cortisol and insulin levels throughout the first three months of life.

Because leptin is a protein derived from adipose tissue and is correlated with the thickness of subcutaneous adipose tissue, we measured the thickness of the subcutaneous adipose tissue of all babies by an ultrasound transducer during their 90 days follow-up. There was a significant difference between preterm and full-term babies at all three measurements. On the other hand, the effect of adipose tissue differed at the three periods. While body weight was the major determinant of serum leptin levels at the 7th day, subcutaneous adipose tissue had a greater determination on leptin concentrations than weight at 30th and 90th days. Girls have a greater adipose tissue thickness than boys at prepubertal age and a higher serum leptin level[10]. But there was no relationship between the thickness of adipose tissue and gender in our study. Similar results were reported by Hytinantti et al.[11], who hypothesized that factors other than adiposity, such as hormonal milieu, may interfere with the association between leptin and adiposity.

Subcutaneous adipose tissue, which is one of the most important sources of leptin, increases in thickness during the first three months of life. But its thickness does not seem to have a great effect on the serum leptin level at the 7th day. In contrast, it is the major determinant of the serum leptin concentrations at the 30th and 90th days of life. This may be a result of the increase in subcutaneous adipose tissue especially after the neonatal period.

Sex-based differences of serum leptin concentrations in postnatal life have been documented extensively. It has been reported that serum leptin levels are higher in female neonates than males[6]. This gender difference was also present in our study at the postnatal 7th and 30th days, but not at the 90th day. Whether the cause of this difference is a suppression of leptin synthesis by androgens remains unclear[6].

The rate of growth is highest in late intrauterine life and early postnatal life. Premature infants may not catch-up with full-term infants early for this reason[12]. Sauve et al.[13] reported that premature infants could not catch-up with full-term infants until their second year of life and that prematures especially had difficulty in increasing their adipose tissue. Smilarly in our study, premature infants could not show an increase in growth and leptin levels like the full-term infants in a 90-day period, but premature babies did show a metabolic catch-up after 90 days.

Recent studies have reported that breast-fed infants have higher body weights and higher serum leptin levels[14]. We suggested breast-feeding for all premature and full-term infants, and because of this fact, we could not research the effect of different diets on serum leptin levels. It could be useful to research the relation between the type of diet and the serum leptin of infants throughout their three months. Leptin has an important role in increasing the energy reserves and in the growth and development of the child. Studies including greater numbers of infants to determine the correlation between increase in weight and adipose tissue and leptin levels have to be carried out.

REFERENCES

1. Stephenss TW, Basinski M, Bristow PK, Bue Valleskey JM, Burgett SG, Craft L. The role of neuropeptide Y in the antiobesity action of the obese gene product. Nature 1995; 377: 530-532.
2. Jaquet D, Leger J, Levy-Marchal C, Oury JF, Czernichow P. Ontogeny of leptin in human fetuses and newborns: impact of intrauterine growth retardation on serum leptin concentrations. J Clin Endocrinol Metab 1998; 83: 1243-1246.
3. Ertl T, Funke S, Sarkany I, et al. Postnatal changes of leptin levels in full-term and preterm neonates: their relation to intrauterine growth, gender and testosterone. Biol Neonate 1999; 75: 167-176.
4. Harigaya A, Nagashima K, Nako Y, Morikawa A. Relationship between concentration of serum leptin and fetal growth. J Clin Endocrinol Metab 1997; 82: 3281-3284.
5. Marchini G, Fried G, Östund E, Hagenas L. Plasma leptin in infants: relations to birth weight and weight loss. Pediatrics 1998; 101: 429-432.
6. 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 1999; 51: 199-204.
7. Ng PC, Lam CWK, Lee CH, et al. Leptin and metabolic hormones in preterm newborns. Arch Dis Child Fetal Neonatal Ed 2000; 83: F198-F202.
8. Koskelo E-K, Kivisaari LM, Saarinen UM, Siimes MA. Quantitaion of muscles and fat by ultrasonography: a useful method in the assessment of malnutrition in children. Acta Paediatr Scand 1991; 80: 1-6.
9. Matsuda J, Yokita I, Iida M, et al. Dynamic changes in serum leptin concentrations during the fetal and neonatal periods. Pediatr Res 1999; 45: 71-75.

10. Hassink SG, Sheslow DV, Lancey E, Opentanova I. Serum leptin in children with obesity: relationship to gender and development. Pediatrics 1996; 98: 201-203.
11. Hytinantti T, Koistinen HA, Koivisto VA, Karonen SL, Andersson S. Changes in leptin concentration during the early postnatal period: adjustment to extrauterine life? Pediatr Res 1999; 45: 197-201.
12. Gibson AT, Carney S, Cavazzoni E, Wales JK. Neonatal and postnatal growth. Horm Res 2000; 53 (Suppl): 42-49.
13. Sauve RS, Geggie JH. Growth and dietary status of preterm and term infants during the first two years of life (Abstract). Can J Public Health 1991; 82: 95-100.
14. Lönnerdal B, Havel PJ. Serum leptin concentrations in infants: effect of diet, sex and adiposity. Am J Clin Nutr 2000; 72: 484-489.