Short communication. Serum leptin levels in obese children and adolescents: relationship to age, gender, body mass index and lipid metabolism parameters

Nivelele serice de leptină la copii şi adolescenţi obezi: relaţia cu vârsta, sexul, indexul de masă corporală şi parametrii metabolismului lipidic

Oana Mărginean[1*], Ana-Maria Pitea[1], Klara Brânzaniuc[2], Adina Huţanu[3], S. Voidăzan[4]

1. University of Medicine and Pharmacy Tg.- Mureş, Pediatric Clinic I
2. University of Medicine and Pharmacy Tg-Mureş, Department of Anatomy
3. County Emergency Hospital Mureş
4. University of Medicine and Pharmacy Tg-Mureş, Department of Epidemiology

Abstract

The aim of the study was to analyze the relationship between leptin serum levels, body mass index and certain parameters of lipid and carbohydrate metabolism in children and adolescents with various degree of obesity. We assessed 86 children and adolescents (45 boys and 41 girls) between 6 and 18 years of age within the Ist Pediatric Clinic from Târgu Mureş; 68.60% of the children were overweight, 29.07% had class I obesity and 2.33% had class II obesity. Leptin serum levels were measured and compared with BMI, age and parameters of carbohydrate and lipid metabolism (blood glucose, cholesterol, triglycerides, HDL cholesterol, and LDL cholesterol). Leptin serum levels were increased in all overweight and obese adolescents: we found leptin serum levels of 45.69 ± 28.04 ng/mL in boys, and significantly higher values (72.96 ± 22.02 ng/mL) in girls. These values were positively correlated with BMI (r = 0,34 in boys and r = 0,44 in girls). A positive correlation was found between leptin, total cholesterol and LDL-cholesterol levels, in boys. No correlations were found between leptin and glycemia, triglycerides and HDL-cholesterol levels. Cholesterol, HDL- and LDL-cholesterol concentrations, as expected, were higher in mothers as compared to their children. The presence of high leptin concentrations in adolescents and children could indicate a predisposition to obesity in adult life.

Keywords: *Obesity, children, leptin*

Rezumat

Scopul studiului a fost studierea relaţiei între nivelele de leptină serică, indexul de masă corporală şi

[*]**Corresponding author :** Marginean Oana, University of Medicine and Pharmacy Tg. Mureş, Pediatric Clinic I, Gh. Marinescu Street No. 38, Tel. 0723278543

parametrii metabolismului lipidic şi glucidic la copiii şi adolescenţii cu diferite grade de obezitate. Au fost investigaţi 86 de copii şi adolescenţi (45 de băieţi şi 41 de fete) cu vârste cuprinse între 14 şi 18 ani, din Clinica de Pediatrie I Târgu Mureş; 60,68% dintre copii au fost supraponderali, 29,07% au avut obezitate clasa I,şi 2,33% au avut obezitate clasa II. Nivelele serice de leptină au fost fost comparate cu vârsta, BMI, parametrii metabolismului glucidic şi lipidic,(glicemie, colesterol, trigliceride, HDL colesterol, and LDL colesterol). Nivelele serice de leptină au fost crescute la toţi adolescenţii obezi şi supraponderali; nivelele serice de leptină au fost 45.69 ± 28.04 ng/mL băieţi, şi semnificativ mai mari la fete(72.96 ± 22.02 ng/mL). Aceste valori au fost pozitiv corelate cu BMI.(r = 0,34 la băieţi şi r = 0,44 la fete). O corelaţie pozitivă a fost observată între leptină si colesterolul total şi LDL colesterol la băieţi. Nu a fost observată nici o corelaţie între nivelele de leptină, trigliceride şi HDL colesterol. Concentraţiile de colesterol total, HDL colesterol, LDL colesterol au fost mai mari la mame comparativ cu copii lor. Concentraţiile crescute de leptină la copii şi adolescenţi pot indica o predispoziţie spre obezitate la vârsta adultă.

Cuvinte cheie *leptină, obezitate, copii*

Introduction

Obesity is a disorder of the nutrition status characterized by excessive accumulation of fat in the subcutaneous cellular tissue or in other tissues and organs as a result of disorders in energy balance. Etiology is multifactorial and involves interactions between genetic, social, behavioral, cellular and molecular aspects, involving changes in energy balance (1). Diagnosis of overweight and obesity uses the assessment of Body Mass Index (BMI) which is defined as the ratio of weight (in kilograms), and height (in square meters) (2, 3). World Health Organization classifies obesity in adults in four subgroups (3), based on BMI (*Table 1*): BMI 25 to 30 kg/m^2 - overweight, BMI 30 to 35 kg/m^2 - grade 1 (moderately obese), BMI 35 to 40 kg/m^2 - grade 2 (severely obese) and BMI > 40 kg/m^2 - grade 3 (morbidly obese). As far as it concerns children, there was no consensus on a cut-off point for excess fatness of overweight or obesity (4, 5).

Before 1994 the scientific literature on overweight and obesity included a wide range of defining criteria (eg, percent ideal weight, skin fold thickness, ponderal index, BMI) and many descriptive names to refer to the children and adolescents who were considered the fattest; the differences in terminology were sometimes confusing (5). Children or adolescents with a BMI at ≥85th percentile but <95th per-

Table 1. The classification of obesity by BMI (adapted after reference 2)

Nomenclature for BMI Ranges			
Classification	Adults	Children and adolescents (CDC, AAP)	Children and adolescents (IOM, IOTF)
Normal range	18.5 – 24.9	5th – 85th percentile	5th - 85th percentile
At risk for overweight		85th - 95th percentile	
Overweight	25.0 – 29.9	≥95th percentile	85th - 95th percentile
Obesity	≥30		
Class I obesity	30.0 – 34.9		
Class II obesity	35.0 – 39.9		
Class III obesity	≥ 40		

AAP - American Academy of Pediatrics; BMI =Weight (kg)/Height (m^2); CDC - Centers for Disease Control; IOM - Institute of Medicine; IOTF - International Obesity Task Force.

centile were considered at risk of overweight. At that time, the term "obese" was avoided, because obesity was technically defined in terms of body fat *per se*, and BMI was derived only from height and weight. In 2005, the Institute of Medicine (IOM) consciously departed from the terminology discussed above and elected to define children with a BMI at ≥95th percentile for age and gender as obese rather than overweight (2). A recent expert committee recommended to replace the terms "at risk of overweight" and "overweight" with the terms "overweight" and "obese," respectively (6, 7). Accordingly, the expert committee recommended that individuals 2 to 18 years of age with a BMI of >30 kg/m^2 or ≥95th percentile for age and gender (whichever is lower) should be considered obese (5, 8).

Overall prevalence of obesity in children has increased worldwide in the last 20 years, reaching an alarming rate. In the USA obesity rates increased by 2.7 - 3.8 times in the last 29 years and in England by 2 - 2.8 times, as revealed by a study done in the past 10 years. After processing data from several countries, experts estimate that there are approximately 22 million obese children aged younger than 5 years worldwide (1, 9, 10).

Risk factors for developing obesity in children are: high weight at birth, maternal obesity during pregnancy, maternal diabetes, obese parents, excess food (hypercaloric, hyperlipidic, hyperglucidic food). Children with BMI over 30 kg/m^2 and those with a rebound of obesity at 5 - 6 years of age have high risk of developing obesity at adult age (3).

Increased levels of triglycerides and cholesterol were observed in obese children with a BMI >30 kg/m^2 (2).

Ghrelin, cholecystokinin, Y peptide, as well as leptin and adiponectin produced by adipocytes have an important role in regulating body mass and determining obesity (3). Leptin is a proteohormone with a molecular weight of 16 kDa, produced by adipocytes in white adipose tissue, encoded by Ob Lep gene on chromosome 7 (11); it plays a particular role in suppressing appetite and regulating body weight (2, 12). The leptin receptor is a member of the class I cytokine receptor family (13, 14). Receptors for leptin are spread throughout the body (15). Acting on the specific receptors in hypothalamus nuclei it plays a key role in regulating energy intake (appetite and metabolism) (2, 11, 15, 16). Leptin influences many endocrine axes (3, 13). In male mice, it blunted the starvation-induced marked decline of LH, testosterone, and thyroxine, and the increase of ACTH and corticosterone. In female mice, leptin prevented the starvation-induced delay in ovulation (15, 17). These effects can be explained by the suppression produced by leptin on neuropeptide Y, secreted by NPY neurons in the arcuate nucleus (18). Further, it is evident that leptin is antagonistic to NPY (19, 20), which is a powerful stimulator of appetite and is known to be incriminated in the regulation of pituitary hormones: suppression of gonadotropins or stimulating the adrenal pituitary axis (18 - 20). The most important variable that determines circulating levels of leptin is body fat mass, with serum leptin reflecting the proportion of adipose tissue (12, 21). Children with obesity can be more specifically classified in subjects with low leptin levels (relative or absolute leptin deficiency) and subjects with high leptin levels (potentially resistant to leptin) (15).

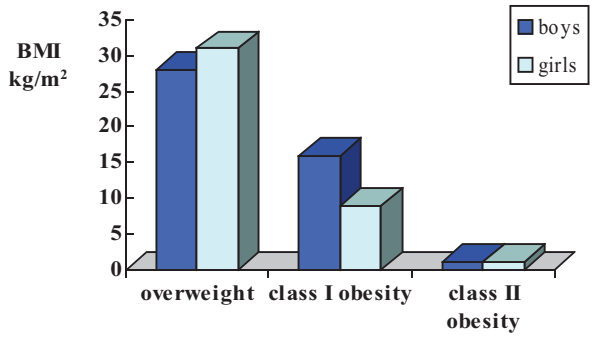

Figure 1. Classification of obesity according to BMI

Besides primary leptin deficiencies, undernutrition can be also a cause for decreased serum levels of leptin. Increased levels are found in obesity and leptin receptor defect.

Discovery of leptin has induced great hopes for effective treatment of obesity. Such hopes subsided when it was discovered that obese people do not respond to leptin because of a central-type resistance to it. Current research has established that some chemical chaperones (4-phenyl butyric acid and tauroursodeoxycholic acid) which have the ability to decrease endoplasmic reticulum stress, sensitize the brain to leptin (22).

Evaluation of children with obesity involves usually the assessment of the existence of dyslipidemia, steatohepatitis or glucose intolerance. Therefore it is necessary to analyze the concentration of cholesterol, triglycerides, very low density lipoproteins, high density lipoproteins and blood glucose.

Objective

The aim of our study was to find relationships between BMI, serum levels of leptin and lipid metabolism parameters (cholesterol, triglycerides, HDL-cholesterol) in overweight and obese children and adolescents.

Subjects and methods

Between September 2005 and December 2008, 86 children and adolescents aged 6 to 18 years, who either were overweight or presented different degrees of obesity, were investigated in Ist Pediatric Clinic in Târgu Mureş; 45 of them were boys, with age of 16±2 years and 41 were girls with age of 15±3 years. Mothers of the children with obesity, with age of 40±4 years, were also investigated, in order to analyze the relation with children parameters.

All adolescents and their parents gave informed consent for participation in the study. None of the participants had signs of acute illness or chronic disease other than obesity.

Serum levels of leptin, cholesterol, triglycerides, HDL-cholesterol, glycemia, were analyzed and correlated with the clinical diagnosis of obesity. The blood was collected and analyzed after at least 10-12 hours fasting, using a Cobas Integra 400 Plus analyzer with standard methods and reagents from Roche

Table 2. Physiological concentrations for plasma lipids in children and adolescents (23)

Component	Normal range mg/dL	Borderline mg/dL	High mg/dL
Total cholesterol	<170	170-190	≥200
LDL-cholesterol	<110	110-129	≥130
Triglycerides			
0-9 years	<75	75-99	≥100
10-19 years	<90	90-129	≥130
HDL-cholesterol	>45	35-45	<35

Table 3 - Reference range of plasma leptin level (ng/mL)

	Males	Females
Adults (BMI 18-25 kg/m^2)	1.2-9.5	4.1-25.0
Children		
Prepubertal	1.6-10.8	1.7-10.6
Tanner I-III	2.1-11.6	2.6-11.5
Tanner IV-V	3.4-10.2	3.4-13.0

Table 4. Demographic and laboratory parameters for evaluated subjects

	Overweight	Obesity gr I	Obesity gr II
N	59	25	2
Boys (B)	28	16	1
Girls (G)	31	9	1
Age (years)	16±2	16±2	B=18;G=6
Leptin (ng/mL)	53.71±27.34	67.14±28.81	> 100
BMI (kg/m^2)	26.19±1.15	31.51±1.00	B=36.00;G=37.70
Glucose (mg/dL)	83.83±9.9	84.83±6.3	B=87.30;G=84.00
Cholesterol (mg/dL)	154.15±26.78	164.56±29.61	B=191.00;G=127.00
Triglycerides (mg/dL)	93.98±47.46	93.39±58.51	B=64.50;G=66.50
HDL–CHOL mg/dL)	62.39±10.85	60.16±10.28	B=31.00;G=65.00
LDL–CHOL mg/dL)	74.66±24.00	85.80±29.8	B=146.30;G=48.80

Diagnostics. The value for LDL-cholesterol was calculated with the Friedewald formula. The thresholds of normal concentrations of plasma lipids are given in *Table 2*.

Leptin Enzyme Immunoassay Kit (GRG) was used to determine the serum concentrations of leptin. Physiological concentration of leptin in adults and children are presented in *Table 3* (24 - 26).

Statistical analysis

Statistical analysis was performed using the GraphPad (San Diego, CA, USA) software. Data with Gaussian distribution were correlated by linear regression. Parametric data were compared by two-tailed *t*-test and ANOVA test. Results are expressed as mean ± SD, median and range. The correlations between serum leptin concentration and gender, age, BMI, cholesterol, triglycerides, HDL-cholesterol, LDL-cholesterol concentrations and glycemia were examined by linear regression and Pearson product-moment correlation analyses. Multiple regression analysis was used to examine the effect of multiple independent variables (e.g. gender, age, BMI, lipid metabolism parameters and glycemia) on a single dependent variable (e.g. serum leptin concentration). Tests significance was defined at the $p < 0.05$, corresponding to a level of confidence of 95%.

Results and discussions

Distribution of obesity in our group of study is represented in *Figure 1*: from the total of 86 subjects included in the study, we made a comparison between the three groups including 59 overweight adolescents (28 boys and 31 girls, mean age 16±2 years, mean BMI 26.19±1.15 kg/m^2), 25 children (16 boys and 9 girls) with class I obesity (mean age 16±2 years, mean BMI 31.00±1.51 kg/m^2) and 2 children (1 boy, 1 girl) with class II obesity (age 18 for boy and 6 for girl, BMI 36.00 kg/m^2 to boy and 37.70 kg/m^2 to girl). In our group class I of obesity and overweight prevailed, data similar to other studies (1).

Across these three groups, we compared the values of leptin, glycemia, cholesterol, triglyceride, LDL- and HDL-cholesterol concentrations and BMI (data in *Table 4).*

We found no statistically significant differences concerning leptin, glycemia, cholesterol, triglyceride, LDL and HDL-cholesterol levels between the three groups after applying the ANOVA test.

We subsequently performed an analysis of the different parameters for male and female adolescents (*Table 5*). Our study comprised 45 boys and 41 girls. Mean ages in the two groups were similar and comparable: 16±2 years in

Table 5. Descriptive analyses of parameters in boys and girls

Parameters	Boys (45)	Girls (41)	p
Leptin (ng/mL)	45.69±28.04	72.96±22.02	0.0001
BMI (kg/m^2)	28.23±3.01	27.40±2.68	0.18
Age (Years)	16±2	15±3	0.07
Glucose (mg/dL)	83.62±10.74	84.82±6.27	0.53
Cholesterol (mg/dL)	154.84±29.05	160.00±26.88	0.4
Triglycerides (mg/dL)	92.85±53.82	93.48±46.61	0.95
HDL –CHOL (mg/dL)	58.98±10.80	64.03±10.99	0.03
LDL –CHOL (mg/dL)	78.50±28.74	77.03±26.26	0.81

Table 6. Comparisons between BMI and parameters of lipid metabolism in children and their mothers

	Children	Mothers	(t test)	Correlation	
	mean±SD	mean±SD	p	r	p
BMI (kg/m^2)	27.59±2.72	28.76±5.69	0.1460	0.58	0.660
Cholesterol (mg/dL)	158.67±27.30	200.72±32.85	0.0001	0.108	0.411
Triglycerides (mg/dL)	95.96±52.06	100.147±62.35	0.5880	0.46	0.0001
HDL–CHOL (mg/dL)	62.35±10.41	70.39±18.29	**0.0017**	0.304	**0.020**
LDL–CHOL (mg/dL)	77.73±25.13	104.40±35.18	0.0001	0.122	0.361

boys and 15±3 years in girls.

There is evidence that girls have higher leptin levels than boys (6 - 12), as previously reported in obese and non-obese children and adolescents (11, 15, 27). This difference increases through puberty as leptin increases from Tanner stage I to V in girls (11); this might be explained at least in part by the higher testosterone levels in males which have a negative effect on leptin concentrations (11, 15).

We analyzed serum levels of leptin in our groups and relationships with gender, age, BMI, cholesterol, triglycerides. In our study, leptin concentrations are significantly lower in boys than in girls. Our data are similar to results of many studies in the literature (11, 27, 28), but some authors have obtained similar data only in subjects over 20 years old (13). In girls, the values are higher, probably due to gender-specific hormonal changes (FSH, LH etc.) (27). The lower values of leptin levels in boys noticed in our study are similar to those in the literature; they increase with age until the age of 10 (11, 27). Leptin levels in boys seem to decrease in parallel with the increase of testosterone levels (27).

From our data we observe that leptin levels are increasing linearly with BMI in both genders (in boys: $y = 0.0398x + 26.423$, $r = 0.34$, $p = 0.012$; in girls, $y=0.0536x+23.503$, $r = 0.44$ and $p = 0.0041$). Three of the children (one boy and two girls, age 9, 11 and 12, respectively) had extremely high leptin concentrations, even though their BMI were below 25 kg/m2. The presence of high leptin values that are independent from the quantity of adipose tissue could indicate an increase in resistance to leptin, a fact that predisposes children to obesity in adult life (12).

The relation between leptin and age in our groups showed a negative correlation (in boys $y= - 0.0475x + 18.565$, $r = -0.55$, $p = 0.0001$; in girls $y = -0.0403x + 17.951$, $r = -0.35$, $p = 0.0248$); these results are different from those related in recent literature (13, 27).

We will carry out further studies on the correlation of leptin values with age, hormonal profile, BMI and dietary habits, on larger groups.

HDL-cholesterol serum levels showed a statistically significant difference between the two groups (boys and girls): the mean value of this parameter was higher in girls than in boys.

We identified a positive correlation between leptin and cholesterol levels ($y=0.5337x+130.44$, $r = 0.51$, $p = 0.0003$) and between leptin and LDL-cholesterol concentrations ($y = 0.602x + 50.716$, $r = 0.59$, $p = 0.0001$) in male. No correlations were observed between leptin and glycemia, HDL-cholesterol or triglyceride levels.

Slightly positive but not statistically significant correlations between leptin and cholesterol, triglycerides or LDL-cholesterol levels were noted in females, whereas the association between leptin and HDL-cholesterol levels was negative, but also not statistically significant. There was no correlation between leptin levels and glycemia in females.

We compared BMI, cholesterol, triglycerides, HDL- and LDL-cholesterol concentrations between children and their mothers. This comparison was carried out only in 64 children-mother pairs comprised in our study, by performing a paired Student test (*Table 6*).

There were no differences between BMI in group of children and mothers but we did not find any correlation between values found in children and their mothers; obesity of the mothers was not associated with obesity of their children. It is well known that the lipid metabolism parameter are higher in adults than children; in our groups total cholesterol, HDL- and LDL-cholesterol levels were significantly higher in mothers than in their children, as expected, and without correlations between cholesterol and LDL-cholesterol levels in mothers and their children. Mean values of HDL-cholesterol concentrations were significantly lower in children than in their respective mothers, with a significant positive correlation between them. Triglyceride levels were insignificantly higher in mothers, but there is a significantly positive correlation with the triglyceride concentrations in their children.

Leptin serum levels were increased in all overweight and obese adolescents and have a positive correlation with BMI, independently of gender. We found a distinct gender difference of serum leptin concentrations: in boys leptin levels are lower than in girls. Leptin levels are positively correlated with cholesterol and LDL-cholesterol concentrations in male children. No correlations were observed between leptin and glycemia, HDL-cholesterol and triglyceride levels. The presence of high leptin concentrations in adolescents and children could indicate a predisposition to obesity in adult life.

References

1. Tarcea M, Dragoi S, Jeszensky K, Dunca I, Toma F. Parametrii serologici si somatometrici la un lot de copii obezi din Targu Mures. Rev. Rom Med. Lab. 2006; 2:71-77
2. Hoppin AG. Evaluation and Management of Obesity. In Duggan C, Watkins JB, Walker AW. Nutrition in Pediatrics. Hamilton, BC Becker Inc 2008;441-450
3. Lustig RH, Weiss R. Disorders of Energy Balance, In Sperling MA. Pediatric Endocrinology. Pittsburgh, Saudders Elsevier 2008;788-838
4. Dehghan M, Danesh NA, Merchant AT. Childhood Obesity, prevalence and prevention Nutr. Jour 2005;4:24
5. Flodmark CE, Lissau I, Moreno LA, Pietrobelli A, Widhalm K: New insights into the field of children and adolescents' obesity: the European perspective International Journal of Obesity 2004, 28, pg 1189.
6. J. H. Himes. Challenges of Accurately Measuring and Using BMI and Other Indicators of Obesity in Children. Pediatrics, September 1, 2009; 124 (Supplement_1): S3 - S22.
7. Krebs NF, Himes JH, Jacobson D, Nicklas TA, Guilday P, Styne D. Assessment of child and adolescent overweight and obesity. Pediatrics. 2007; 120(suppl 4): S193–S228
8. Barlow S; Expert Committee. Expert Committee recommendations regarding the prevention, assessment, and treatment of child and adolescent overweight and obesity: summary report. Pediatrics. 2007;120(suppl 4):S164–192
9. De Wieland K, Marcus C, Wabitsch M. Obesity in childhood and adolescence. Karger, Basel 2004, p171.
10. Deckelbaum RJ, Williams CL. Childhood obesity: the health issue. Obes Res. 2001 Nov; 9 Suppl 4:239S-

243S.
11. Antunes H, Santos C, Carvalho S. Serum leptin levels in overweight children and adolescents. British Journal Nutr 2009, 101; 1262–1266 doi:10.1017/S0007114508055682
12. Fleisch AF, Agarwal N, Roberts MD, Han JC, Theim KR, Vexler A, et al. Influence of Serum Leptin on Weight and Body Fat Growth in Children at High Risk for Adult Obesity. The Journal of Clinical Endocrinology & Metabolism 92(3):948–954. doi: 10.1210/jc.2006-1390
13. Mann DR, Johnson AOK, Gimpel T, Castracane VD. Changes in Circulating Leptin, Leptin Receptor, and Gonadal Hormones from Infancy until Advanced Age in Humans. The Journ Clin Endocrin. & Metab. 2002;88(7):3339–3345; doi: 10.1210/jc.2002-022030
14. Kratzsch J, Deimel A, Galler A, Kapellen T, Klinghammer A, Kiess W. Increased serum soluble leptin receptor levels in children and adolescents with type 1 diabetes mellitus. European Journ Endocrin. 2004, 151 475–481; ISSN 0804-4643
15. Blum WF, Englaro P, Hanitsch S, Juul A, Hertel NT, Müller J, et al. Plasma Leptin Levels in Healthy Children and Adolescents: Dependence on Body Mass Index, Body Fat Mass, Gender, Pubertal Stage, and Testosterone. Jour of Clin Endocrinol & Metab 1997;82:2904-2910
16. MacDougald OA, Hwang CS, Fan H, Lane MD. Regulated expression of the obese gene product (leptin) in white adipose tissue and 3T3–L1 adipocytes. Proc Natl Acad Sci USA. 1995;92:9034–9037
17. Ahima RS, Prabakaran D, Mantzoros C, Qu D, Lowell B, Maratos-Flier E, et al. Role of leptin in the neuroendocrine response to fasting. Nature 1996;382:250–252
18. Schwartz MW, Baskin DG, Bukowski TR, Kuijper JL, Foster D, Lasser G, et al. Specificity of leptin action on elevated blood glucose levels and hypothalamic neuropeptide Y gene expression in ob/ob mice. Diabetes 1996;45:531–535
19. Chan YY, Steiner RA, Clifton DK. Regulation of hypothalamic neuropeptide-Y neurons by growth hormone in the rat. Endocrinol. 1996;137:1319–1325
20. Rohner-Jeanrenaud F, Cusin I, Sainsbury A, Zakrzewska KE, Jeanrenaud B. The loop system between neuropeptide Y and leptin in normal and obese rodents. Horm Metab Res. 1996;28:642–648
21. Ruhl CE, Everhart JE, Ding J, Goodpaster BH, Kanaya AM, Simonsick EM, et al. Serum leptin concentrations and body adipose measures in older black and white adults. Am J Clin Nutr 2004;80:576–83
22. Ozcan L, Ergin AS, Lu A, Chung J, Sarkar S, Nie D, et al. Endoplasmic Reticulum Stress Plays a Central Role in Development of Leptin Resistance. Cell Metabol 2009;9(7):35–51
23. Couch SC, Daniels SR. Lipid Disorders in Children and Adolescents, In Sperling MA. Pediatric Endocrinology. Pittsburgh, Sauders Elsevier 2008;839-854
24. Ma Z, Gingerich RL, Santiago JV, Klein S, Smith CH, Landt M. Radioimmunoassay of leptin in human plasma. Clinical Chemistry 1996;42:(6)942-946
25. Fisher DA (ed.). The Quest Diagnostics Manual Endocrinology Test Selection and Interpretation, fourth Ed. 2007 Quest Diagnostics, USA, p. 122.
26. Clayton PE, Gill MS, Hall CM, Tillmann V, Whatmore AJ, Price DA. Serum leptin through childhood and adolescence. Clin Endocrinol (Oxf). 1997 Jun;46(6):727-33.
27. Garcia-Mayor RV, Andrade M, Rios M, Lage M, Dieguez C, Casanueva FMF. Serum leptin levels in normal children: Relationship to age, gender, body mass index, pituitary-gonadal hormones and pubertal stage. J Clin Endocrinol Metab 2003; 36(10):1293-6.
28. Carlsson B, Ankarberg C, Rosberg S, Norjavaara E, Albertsson-Wikland K, Carlsson LMS. Serum leptin concentrations in relation to pubertal development. Archives of Disease in Childhood 1997;77:396–400