

Serum leptin levels in patients with 21-hydroxylase deficiency before and after treatment

Şükran Poyrazoğlu, Hülya Günöz, Feyza Darendeliler

Department of Pediatrics, İstanbul Univeristy, İstanbul Faculty of Medicine, İstanbul, Turkey

SUMMARY: Poyrazoğlu Ş, Günöz H, Darendeliler F. Serum leptin levels in patients with 21-hydroxylase deficiency before and after treatment. Turk J Pediatr 2003; 45: 33-38.

Increase in serum androgen levels results in suppression of serum leptin levels. In this study, the changes in serum leptin concentrations of children with congenital adrenal hyperplasia (CAH) due to 21-hydroxylase deficiency (21-OHD) with respect to their hyperandrogenism were investigated. Eleven children with 21-OHD and 25 healthy control children were included in the study.

Before initiation of hydrocortisone, serum leptin levels in children with CAH were lower (1.7 ± 1.3 ng/ml) than in the control group (5.3 ± 4.01 ng/ml) ($p < 0.001$). After three months of treatment, serum leptin levels increased to the normal range (7.1 ± 2.9 ng/ml). Prior to and on hydrocortisone treatment in CAH, serum leptin levels were positively correlated with cortisol ($r: 0.78$, $p: 0.004$ and $r: 0.80$, $p: 0.003$) but negatively correlated with testosterone ($r: -0.62$, $p: 0.04$ and $r: -0.65$, $p: 0.002$).

These results suggest that serum leptin measurements may be used as an additional parameter in the follow-up of children with CAH to evaluate the efficacy of hydrocortisone treatment with respect to androgenemia.

Key words: leptin, 21-hydroxylase deficiency.

Leptin, the obese (ob) gene product, is a 16 kDa peptide hormone secreted mainly by adipocytes¹⁻³. This hormone conveys to the brain information about the size of energy stores and activates hypothalamic centers that regulate energy intake and expenditures. Besides regulation of energy balance, leptin appears to influence several neuroendocrine mechanisms and regulates multiple hypothalamic-pituitary hormones³⁻⁵.

Although the regulation and metabolism of leptin in humans and its precise role in the endocrine system are poorly understood, leptin production by adipose tissue is under neuroendocrine control²⁻⁴.

There is a positive correlation between body fat mass and circulating leptin concentrations⁵⁻⁷. Recent studies have shown that insulin and glucocorticoids are positive regulators of leptin. Glucocorticoids increase leptin production in vitro, and exogenously administered glucocorticoids produce a rise in circulating leptin levels in humans. An inhibitory effect has also been reported for androgens^{3,4,8,9}.

The purpose of this study was to determine changes in serum leptin levels in children with congenital adrenal hyperplasia (CAH) due to 21-hydroxylase deficiency with respect to androgenemia.

Material and Methods

We studied 11 children with classical 21-hydroxylase deficiency (5 girls, 6 boys) with a mean (SD) age of 1.6 (2.7) years from the pediatric endocrine clinic and 25 healthy children (13 girls, 12 boys) aged 1.5 (2.4) years as the control group. Informed consent was obtained from each family.

Regarding the initial symptoms, four girls presented with ambiguous genitalia, one girl with hirsutism and six boys with scrotal hyperpigmentation. One girl and three boys had additional symptoms of salt loss.

Of the total 11 children with CAH, seven of the patients were newly diagnosed patients whose blood samples for basal hormone measurements

were taken at the time of diagnosis prior to initiation of glucocorticoid therapy. Four of the patients were already on treatment. These patients were admitted to the ward and their hydrocortisone treatment was discontinued for five days under close observation. Blood samples for "before treatment" measurements were taken at the end of this five-day-off therapy. Hydrocortisone was reinstated afterwards.

All patients were treated with hydrocortisone in a mean dose of 14.6 ± 4 mg/m²/day and five patients also with fludrocortisone in a mean dose of 0.04 ± 0.01 mg/day because of overt salt loss or high plasma rennin activity (PRA). Blood samples for "after treatment" hormone measurements were taken 4.1 (2.6) months after initiation of hydrocortisone treatment. All the samples were collected after an overnight fast between 08:00 and 09:00 hours before the hydrocortisone dose.

Serum leptin, cortisol, testosterone, 17-hydroxyprogesterone (17-OHP) and androstenedione (AS) were measured in CAH patients before and after treatment and serum leptin and testosterone in the controls. After centrifugation, serum samples were immediately stored at -20°C until later analysis.

Height was measured using Harpenden stadiometer and weight was determined to the nearest 0.1 kg using a calibrated scale before and after treatment in CAH patients and in the controls. Body mass index (BMI) calculated as weight (kg)/height (m)² was used as an index of overall adiposity. BMI standard deviation scores were calculated according to the standards of Rolland-Cachera et al.¹⁰.

Serum leptin was measured by immunoradiometric assay (Diagnostic Systems Laboratories, Inc, Texas). The lower limit of detection was 0.10 ng/ml. Intra- and inter-assay coefficients of variation were 3.7% and 5.2%, respectively.

Androstenedione (AS) and 17-OHP were measured by radiimmunoassay (Diagnostic Systems Laboratories, Inc, Texas). The lower limits of detection for AS and 17-OHP were 0.03 ng/ml and 0.01 ng/ml, respectively. For AS intra- and inter-assay coefficients of variations were 4.2% and 7.6%, respectively and for 17-OHP 8.9% and 8.9%.

Chemiluminescence was used for measurement of testosterone and cortisol (Chirion Diagnostics ACS). The lower limit of detection for testosterone and cortisol was 10 ng/dl and 0.20 µg/dl, respectively. For testosterone intra- and inter-assay coefficients of variations were 6.8% and 8.6%, respectively and for cortisol 6% and 8.4%.

Differences between groups were tested using Mann-Whitney U test for unrelated samples and Wilcoxon test for paired samples. Correlations were made with Pearson's test. Results were expressed as mean \pm SD. A value of $p < 0.05$ was considered statistically significant.

Results

Before initiation of treatment, serum cortisol levels of four patients were lower than the normal range. On hydrocortisone substitution therapy, cortisol levels increased and 17-OHP and AS levels decreased in all patients (Table I).

Table I. BMI and Hormonal Levels of Patients and Controls

	Patients with 21-OHD		p*	Control group (n=25)
	Before treatment (n=11)	After treatment (n=11)		
BMI (kg/m ²)	13.5 (2.1)	16.4 (1.5)	NS	16.1 (1.7)
Leptin (ng/ml)	1.7 (1.3)	7.1 (2.9)	0.003	5.3 (4.0)
Testosterone (ng/dl)	148.6 (157.2)	4.0 (6.5)	0.003	34.5 (48.3)
Cortisol (µg/dl)	5.5 (2.52)	8.6 (2.8)	0.004	–
AS (ng/ml)	5.6 (4.5)	0.2 (0.2)	0.003	–
17-OHP (ng/ml)	64.9 (144.1)	3.1 (4.0)	0.005	–

* Wilcoxon. Comparisons were made between before and after treatment values.

Values represented as mean (SD).

BMI : body mass index.

AS : androstenedione.

21-OHD : 21-hydroxylase deficiency.

17-DHP : 17-hydroxyprogesterone.

Before treatment, leptin levels were significantly lower ($p=0.001$), but testosterone levels were significantly higher ($p=0.006$) than that of the age- and sex-matched control group (Table I). After hydrocortisone replacement therapy, leptin levels increased and testosterone levels decreased to the normal range in CAH patients.

Leptin was significantly and positively correlated with cortisol before ($r=0.78$, $p=0.004$) and after ($r=0.80$, $p=0.003$) treatment but negatively correlated with testosterone before ($r=-0.62$, $p=0.04$) and after ($r=-0.65$, $p=0.02$) treatment (Fig. 1). Neither leptin nor cortisol levels correlated significantly with 17-OHP or AS levels before or after treatment.

Leptin levels before ($r=0.78$, $p=0.004$) and after ($r=0.82$, $p=0.002$) treatment in CAH patients and in controls ($r=0.67$, $p=0.001$) were positively and significantly correlated with BMI.

In the control group, leptin levels of girls (6.8 ± 4.6) were higher than those of boys (3.7 ± 2.5) ($p=0.04$). This gender difference in leptin levels was not evident in children with CAH before or after treatment (respectively, leptin in girls 2.1 ± 1.4 ng/ml, 7.4 ± 3.9 ng/ml; leptin levels in boys 1.3 ± 1.1 ng/ml, 6.9 ± 2.0 ng/ml). BMI did not show a gender difference in either group.

The correlations between hydrocortisone replacement dose and serum levels of 17-OHP, AS, leptin and cortisol were not statistically significant.

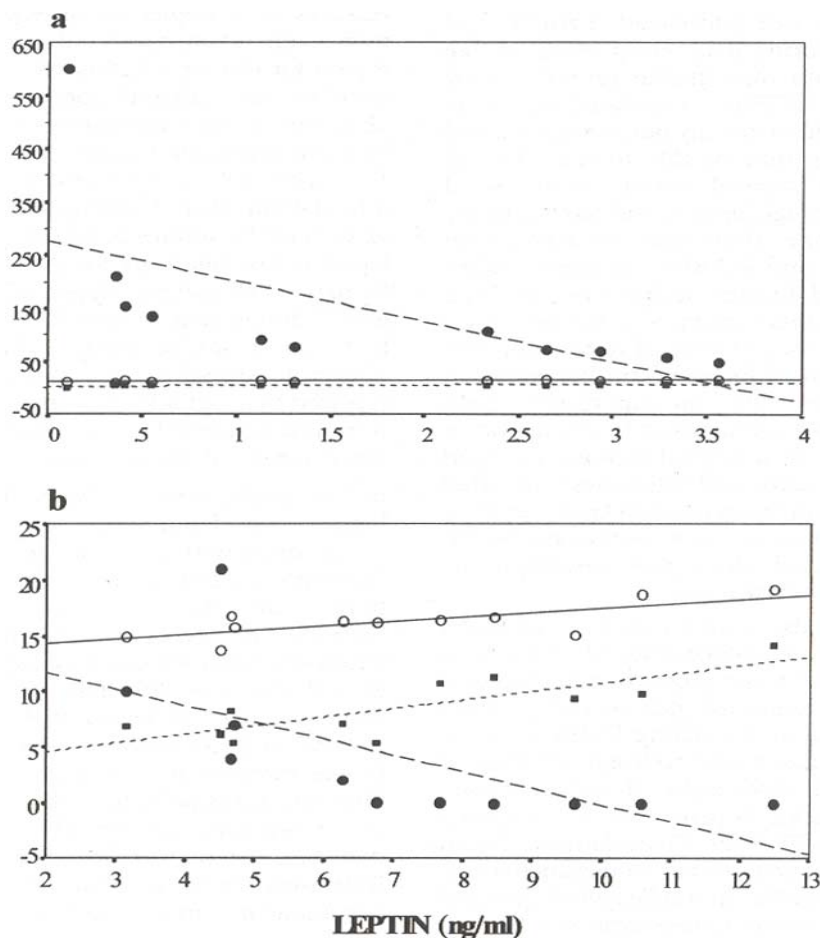


Fig. 1. Leptin plotted against body mass index (BMI) (kg/m^2), testosterone (ng/dl), and cortisol (m/dl) in children with congenital adrenal hyperplasia (CAH) before (a) and after (b) treatment (respectively $r=0.78$, $p=0.004$, $r=0.62$, $p=0.041$, $r=0.78$, $p=0.004$ and $r=0.82$, $p=0.002$, $r=0.65$, $p=0.029$, $r=0.80$, $p=0.003$). Regression lines for testosterone (\bullet and $- -$), cortisol (\blacksquare and $- -$), and BMI (\circ and $-$) are shown.

Discussion

Leptin's physiology and the precise role it plays in the endocrine system remain to be defined. Testosterone might modulate plasma leptin concentration by affecting body fat content and distribution¹⁰⁻¹².

In our study, leptin was found to be negatively correlated with testosterone. Sih et al.¹³ and Hermann et al.¹⁴ demonstrated that the increased levels of leptin in hypogonadal males decreased with testosterone therapy. Touminen and colleagues¹⁵ also found that in insulin dependent diabetics, fasting plasma leptin concentrations were negatively correlated with fasting plasma testosterone concentrations. The mechanism by which testosterone induces a decrease in serum leptin levels is not addressed. Erfurth and colleagues¹⁶ reported that serum levels of free testosterone and insulin-like growth factor binding protein (IGFBP-1) correlated negatively with serum leptin in healthy nonobese men, and this relationship may be due to the effect of IGFBP-1. Blum¹⁷ showed that in boys below 19 years of age, although leptin correlated negatively with testosterone, there was no association between leptin and IGFBP-1. In vitro studies using cultured human adipocytes provide evidence for a direct effect of testosterone on adipose tissue. The presence of testosterone on culture medium reduced leptin secretion in adipocytes up to 62% and suppressed leptin mRNA levels to a similar extent¹². At the onset of puberty there is a marked increase in leptin levels¹⁷⁻²⁰. Mantzoros and colleagues²¹ reported a consistent rise in leptin levels in boys preceding the increase in testicular size. Before peak height velocity is reached, the leptin concentrations return to prepubertal levels.

Glucocorticoids also have a regulatory effect on serum leptin levels. Glucocorticoids have been shown to increase leptin production in vitro, and exogenously administered glucocorticoids produce a sustained rise in circulating leptin levels in humans²²⁻²⁶. Janssen and colleagues²⁴ showed that after an overnight dexamethasone suppression test, leptin levels increased significantly at 9th hour. Posttreatment leptin levels were positively related to dexamethasone levels. Papaspyrou-Rao and colleagues²⁵ reported that short-term corticosteroid treatment induces an increase in fasting leptin levels in humans. Dagogo-Jack and colleagues²⁶ demonstrated that plasma leptin response to dexamethasone treatment was similar in both sexes at all ages.

The hydrocortisone dose our patients were using was within the replacement dose ranges (14 ± 4 mg/m²/day). After initiation of hydrocortisone therapy, both serum cortisol and leptin levels of our patients increased. In agreement with previous studies, strong positive correlation between serum leptin and cortisol levels shows that cortisol has an important role in regulating serum leptin levels.

In vitro studies have shown that the effect of dexamethasone on leptin levels may be due to a direct effect on leptin production in adipose cells by increasing leptin mRNA expression or to the presence of a glucocorticoid responsive element in the promoter of the ob gene².

Females have higher serum leptin levels than men even when corrected for BMI^{11,27}. The reason for this sexual dimorphism is unclear. It could be due to an inhibitory effect of androgens on leptin, or to a difference in fat distribution between males and females, since subcutaneous fat produces more leptin mRNA than intraabdominal fat. Hassink et al²⁸ reported that, as seen in the studies of adults, girls had higher leptin levels than boys for the same amount of fat mass at all pubertal stages. Ellis et al.²⁹, Nagy et al.³⁰ found that in prepubertal children girls had higher serum leptin values than boys. However, Ahmed et al.³¹ and Clayton et al.³² reported that leptin levels in prepubertal children were not different between boys and girls even when corrected for fat mass.

In our study, girls in the control group had higher serum leptin levels than boys. However, in children with CAH there was no sexual dimorphism between boys and girls either before or after treatment. High serum testosterone levels in CAH patients before treatment may have caused disappearance of the sexual dimorphism. The variability in the increase in leptin levels after hydrocortisone therapy in CAH may be due to the variability in the increase in serum cortisol levels with different doses of hydrocortisone. Probably for these reasons, no sexual dimorphism was detected. There was no gender difference in BMI neither in patients before and after treatment nor in the controls.

In the present study, BMIs of our patients were within normal ranges both before and after treatment. During hydrocortisone replacement therapy, BMIs of patients increased.

Similar to previous studies^{12,29,30}, there was a positive significant correlation between leptin levels and BMI in controls and in patients in our study. Hassink and colleagues²⁸ reported that similar to adults, children with high BMI values have higher serum leptin concentrations than children with normal BMI.

The aim of our study was to determine changes in serum leptin levels of children with CAH due to 21-hydroxylase deficiency. Before treatment serum testosterone levels were high and leptin levels were low, but after hydrocortisone treatment testosterone levels decreased and serum leptin and cortisol levels increased. There was a positive correlation between serum cortisol and leptin levels. The correlation between leptin and cortisol was more significant than that between leptin and testosterone.

Although it is obvious that multiple factors play a role in regulating the serum leptin level, glucocorticoids, testosterone and BMI seem to modify leptin levels to a great extent. All these factors interplay with each other in CAH. Uncontrolled hyperandrogenemia is one of the major adverse features in CAH. Since serum leptin and testosterone levels show a remarkable negative correlation, leptin measurement may provide additional information on the degree of androgenemia in the follow-up of these patients. Secondly, provided that BMI is within normal ranges in CAH, leptin may be indicative of effective hydrocortisone treatment since cortisol and leptin are correlated. Leptin may also indicate overdose of hydrocortisone treatment since both BMI and cortisol levels correlate with leptin. Based on the findings obtained in our study, it can be concluded that leptin may be used as an additional parameter in the follow-up of children with CAH.

Acknowledgements

This work was supported by the Research Fund of the University of İstanbul, project number: T-558/240698.

REFERENCES

- 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: 425-432.
- Mantzoros C, Moschos SJ. Leptin: in search of role(s) in human physiology and pathophysiology. *Clin Endocrinol* 1998; 49: 551-567.
- Kiess W, Siebler T, Englaro P, et al. Leptin as a metabolic regulator during fetal and neonatal life and in childhood and adolescence. *J Pediatr Endocrinol Metab* 1998; 11: 483-496.
- Flier JS. Clinical review 94: In search of leptin' physiologic role. *J Clin Endocrinol Metab* 1998; 83: 1407-1413.
- Qureshi A, Kopelman P. Leptin-fat messenger or fat controller. *Clin Endocrinol* 1997; 47: 169-171.
- Friedman JM, Halaas JL. Leptin and regulation of body weight in mammals. *Nature* 1998; 395: 763-770.
- Considine R, Madhur K, Sinha D, et al. Serum immunoreactive-leptin concentrations in normal weight and obese humans. *N Engl J Med* 1996; 334: 292-295.
- Cohen B, Novick D, Rubinstein M. Modulation of insulin activities by leptin. *Science* 1996; 274: 1185-1188.
- Cusin I, Amanda S, Doyle P, Rohner-Jeanrenaud F, Jeanrenaud B. The ob gene and insulin. *Diabetes* 1995; 44: 1467-1470.
- Rolland-Cachera ME, Cole T, Sempe M, Tichet J, Rossignol C, Charraud A. Body mass index variations. *Eur J Clin Nutr* 1991; 45: 13-21.
- Saad M, Damani S, Gingerich RL, et al. Sexual dimorphism in plasma leptin concentration. *J Clin Endocrinol Metab* 1997; 82: 579-584.
- Wabitsch M, Blum W, Muehe R, et al. Contribution of androgens to the gender difference in leptin production in obese children and adolescents. *J Clin Invest* 1997; 100: 808-813.
- Blum W, Englaro P, Hanitsch S, et al. Plasma leptin levels in healthy children and adolescents: dependence on body mass, gender, pubertal stage, and testosterone. *J Clin Endocrinol Metab* 1997; 82: 2904-2910.
- Sih R, Morley J, Kaiser F, Perry IIIrd HM, Patrick P, Ross C. Testosterone replacement in older hypogonadal men: a 12-month randomized controller trial. *J Clin Endocrinol Metab* 1997; 82: 1616-1667.
- Behre HM, Simoni B, Nieschlag E. Strong associations between serum levels of leptin and testosterone in men. *Clin Endocrinol* 1997; 47: 237-240.
- Erfurth EM, Ahren B. The negative association between serum free testosterone and leptin is dependent on insulin-like growth factor-binding protein 1 in healthy young and middle-aged men. *Clin Endocrinol* 2000; 52: 493-498.
- Tuominen JA, Ebeling P, Stenman U, Heiman ML, Stephens TW, Koivisto VA. Leptin synthesis is resistant to acute effects of insulin in insulin-dependent diabetes mellitus patients. *J Clin Endocrinol Metab* 1997; 82: 381-382.
- Apter D. Leptin and puberty. *Curr Opin Endocrinol Diab* 2000; 7: 57-64.
- Rogol A. Editorial: leptin and puberty. *J Clin Endocrinol Metab* 1998; 83: 1089-1090.
- Kiess W, Blum W, Aubert ML. Leptin, puberty and reproductive function: lessons from animal studies and observations in humans. *Eur J Endocrinol* 1998; 138: 26-29.
- Mantzoros C, Flier J, Rogol AD. A longitudinal assessment of hormonal and physical alterations during normal puberty in boys. v. Rising leptin levels may signal the onset of puberty. *J Clin Endocrinol Metab* 1997; 82: 1066-1070.

22. Berneis K, Vosmeer S, Keller U. Effects of glucocorticoids and of growth hormones on serum leptin concentrations in man. *Eur J Endocrinol* 1996; 135: 663-665.
23. Laferrere B, Fried SK, Hough K, Campbell SA, Thornton J, Pi-Sunyer FX. Synergistic effects of feeding and dexamethasone on serum leptin levels. *J Clin Endocrinol Metab* 1998; 83: 3742-3747.
24. Janssen JA, Huizenga NA, Stolk RP, et al. The acute effect of dexamethasone on plasma leptin concentrations and the relationships between fasting leptin, the IGF-I/IGFBP system, dehydroepiandrosterone, androstenedione and testosterone in an elderly population. *Clin Endocrinol* 1998; 48: 621-626.
25. Papaspyrou-Rao S, Schneider SH, Petersen RN, Fried SK. Dexamethasone increases leptin expression in humans in vivo. *J Clin Endocrinol Metab* 1997; 82: 1635-1638.
26. Dagogo-Jack S, Selke G, Melson AK, Newcomer JW. Robust leptin secretory responses to dexamethasone in obese subjects. *J Clin Endocrinol Metab* 1997; 82: 3230-3233.
27. Roemmich J, Clark P, Berr SS, et al. Gender differences in leptin levels during puberty are related to the subcutaneous fat depot and sex steroids. *Am J Physiol* 1998; E543-551.
28. Hassink SZ, Sheslow D, Lancey E, Opentanova I, Considine R, Caro JF. Serum leptin in children with obesity: relationship to gender and development. *Pediatrics* 1996; 98: 201-203.
29. Ellis K, Nicolson M. Leptin levels and body fatness in children: effect of gender, ethnicity, and sexual development. *Pediatr Res* 1997; 42: 4484-4488.
30. Nagy T, Gower B, Trowbridge A, Dezenberg C, Shewchuk RD, Goran MI. Effects of gender, ethnicity, body composition and fat distribution on serum leptin concentrations in children. *J Clin Endocrinol Metab* 1997; 82: 2148-2152.
31. Ahmed M, Ong KK, Morell DJ, et al. Longitudinal study of leptin concentrations during puberty: sex differences and relationship to changes in body composition. *J Clin Endocrinol Metab* 1999; 84: 899-905.
32. Clayton P, Gill M, Hall C, Tillmann V, Whatmore A, Price D. Serum leptin through childhood and adolescence. *Clin Endocrinol* 1997; 46: 727-734.