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Leptin Levels in Obese Children: Effects of Gender, Weight Reduction and Androgens

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Summary

Obesity in children is accompanied by increased circulating leptin concentrations. Girls have higher leptin concentrations than boys. The aim of our study was to compare serum leptin levels before and after a five-week weight reduction program and to study the relationship of leptin levels, serum total cholesterol, and androgens (testosterone, dehydroepiandrosterone, dehydroepiandrosterone sulphate) in 33 obese boys (age: 12.7±1.97 years, BMI: 30.46±4.54) and 66 obese girls (age: 12.7±2.51 years, BMI: 29.31±4.62). We found that serum leptin concentrations in obese children were significantly decreased after a weight reduction program (before 20.79±9.61 ng/ml, after 13.50±8.65 ng/ml in girls; before 12.25±10.09 ng/ml and after 5.18±3.56 ng/ml in boys, $p<0.0001$ in both genders). Leptin levels correlated positively with the body mass index before and after weight reduction. There was a positive association in obese boys and a negative one in obese girls between leptin levels and the WHR (waist to hip circumference ratio). Serum leptin also shows a strong relationship to fat distribution ($p = 0.02$ in boys, $p<0.0001$ in girls). No significant correlation was found between leptin concentrations and total cholesterol or androgens. We confirmed that leptin is a sensitive parameter of body composition and weight reduction in obese children.

Key words
Leptin • Children • Obesity • Androgens

Introduction

Childhood obesity has been associated with morbidity and mortality in adulthood independent of adult weight and other risk factors (Must *et al.* 1992). The identification of obesity risk factors and their role in the development of excess fat depots in childhood is therefore necessary in order to plan more successful strategies. Since obesity is a multifactorial disease and its development is the result of multiple interactions between genes and the environment, both genetic and environmental components have to be investigated to detect more sensitive targets for medical intervention.

The ob gene that produces leptin was identified by Zhang *et al.* (1994). Leptin is a specific protein, which is secreted mostly by white adipocytes into the circulation and may represent an important afferent signal in the brain (Klein *et al.* 1996). An association between body weight regulation and the neuroendocrine system was proposed many years ago (Hetherington and Ranson

1940). Leptin regulates the expression of hypothalamic neuropeptides involved in the regulation of feeding and neuroendocrine functions (Ahima *et al.* 2000). Haluzík *et al.* (1999a,b,c) referred low serum leptin levels in patients with anorexia nervosa. On the contrary, obesity in adults (Considine *et al.* 1996) and children (Reiterer *et al.* 1999) is accompanied by increased circulating leptin concentrations. Leptin correlates most significantly with BMI and body fat (Argente *et al.* 1997). Several studies have confirmed that serum leptin levels decrease following weight reduction (Considine *et al.* 1996, Reiterer *et al.* 1999).

It has been shown that leptin levels are higher in females as compared with males even after correction for the degree of body fat mass (Hassink *et al.* 1996, Rosenbaum *et al.* 1996). Martin *et al.* (2002) showed convincing evidence that different genes influence variations in serum leptin levels between the two sexes. Furthermore, this pattern of sexual dimorphism was eliminated after accounting for the effects of testosterone. Sinha *et al.* (1996) proposed that androgens have a suppressive effect on serum leptin levels. Testosterone treatment reduces serum leptin concentrations in adolescents with delayed puberty (Arslanian and Suprasongsin 1997) as well as in hypogonadal (Jockenhovel *et al.* 1997) and eugonadal men (Luukkaa *et al.* 1998). Nedvídková *et al.* (1997) have shown that this difference is probably not estrogen-dependent. The gender difference becomes evident in early puberty in conjunction with developing dimorphism in sex steroid production (Wabitsch *et al.* 1997).

Plasma concentrations of dehydroepiandrosterone (DHEA) in adults are inversely related to age (Šulcová *et al.* 1997) and decrease in association with increased incidence of diabetes mellitus, obesity and atherosclerosis (Poršová-Dutoit *et al.* 2000). It has been hypothesized that the decline in DHEA levels with advancing age in humans plays a role in the development of visceral obesity and insulin resistance (De Pergola 2000).

The aims of our study were: 1) to investigate the effects of a five-week intervention program including low caloric diet and supervised physical training on body composition in obese children, and 2) to investigate a possible sexual dimorphism of leptin in response to androgens during the puberty.

Methods

We studied 99 children, 33 boys (age: 12.7±1.97 years, BMI: 30.46±4.54 kg/m^2), and 66 girls (age: 12.7±2.51 years, BMI: 29.31±4.62 kg/m^2) before and after a five-week weight reduction program. All anthropometric and metabolic characteristics are given in Table 1. All children were judged healthy by history and physical examination. Written informed consent was given by the parents of the children. Sexual maturation was examined using Tanner stages at the beginning of the study. BMI was calculated as a measure of the degree of obesity. All children had a BMI greater than 85 % percentile specific for age and gender for the Czech population (Vignerová and Bláha 2001). Subscapular, triceps and suprailiac skinfold thicknesses were measured using Best's calliper (Martin and Saller 1957). The waist circumference was measured at the umbilicus and hip circumference at the widest point to the nearest 0.5 cm in all children. Blood samples were taken after an overnight fast before measurements of body composition, at the beginning and after 5 weeks of the intervention program. After centrifugation, serum samples were immediately stored at – 20 °C until later analysis. Commercially available test kits were used to measure leptin (ELISA method, DRG International, USA), DHEAS, DHEA (Immunotech, France). Testosterone concentrations were measured by a RIA method using home kits (Hampl *et al.* 1993). Children were assigned to a mixed diet of 5000 kJ per day in children under 10 years old and 7000 kJ per day in children older than 10 years. Supervised physical activities including biking, swimming, and various ball games were performed for 3 h every day.

For normally distributed data, standard statistics (Student´s t-test and Pearson correlation coefficients) were used for analysis. For data not normally distributed, non-parametric statistics were employed. A two-way ANOVA was performed to detect influences of sex on leptin concentrations. $P<0.05$ values were considered significant. All values are means ± S.D.

Results

Serum leptin levels significantly correlated with BMI in both genders (Fig. 1). Leptin concentrations were higher in girls (20.79±9.61 ng/ml) than in boys (12.25±10.09 ng/ml, $p<0.001$). However, even after correcting for BMI the gender difference in leptin levels remains significant ($p = 0.02$). In the group of obese girls, there were strong positive associations between serum leptin concentrations and age, all skinfold thicknesses, hip and waist circumference, whereas the association with WHR was negative. No associations were obtained between serum leptin and other parameters such as total cholesterol, testosterone, DHEA or DHEAS (Table 1).

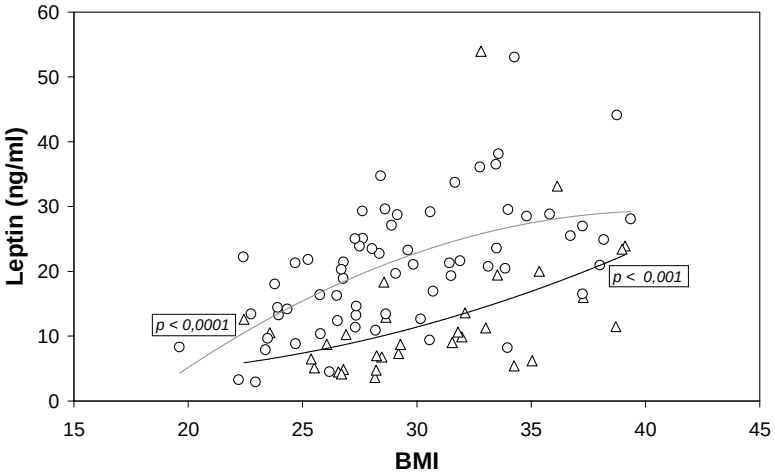

Fig. 1. *Leptin levels in obese girls (○, dotted line, n=66) and obese boys (Δ, full line, n=33) in relation to BMI (T-test), significant sexual difference p<0.0001 (ANOVA test).*

In obese boys, we found significant relationships between serum leptin levels and the WHR, suprailiac, and subscapular skinfold thicknesses (Table 1). We did not observe any correlation between serum leptin and total cholesterol, testosterone, DHEA, and DHEAS (Table 1).

Table 1. Physical and metabolic characteristics of obese boys and obese girls at the beginning of the study.

	Obese girls (n = 66)			**Obese boys** (n = 33)		
	Mean ± SD	**r**	**p**	**Mean ± SD**	**r**	**p**
Leptin (ng/ml)	20.79±9.61	–	–	12.25±10.09	–	–
Age (years)	12.7±2.51	0.4084	**0.001**	12.7±1.97	0.2620	0.1380
BMI (kg/m^2)	29.31±4.62	0.5919	**0.0001**	30.46±4.54	0.6177	**0.0005**
Waist circumference (cm)	91.27±9.76	0.5380	**0.0001**	98.78±11.49	0.6660	**0.0002**
Hip circumference (cm)	102.12±11.48	0.5112	**0.0001**	101.33±9.95	0.5419	**0.0022**
WHR	0.89±0.06	–0.5043	**0.0001**	0.97±0.05	0.4340	**0.0141**
Suprailiac skinfold (mm)	25.35±6.24	0.5820	**0.0001**	29.03±5.66	0.4945	**0.0052**
Triceps skinfold (mm)	24.97±5.36	0.5190	**0.0001**	24.14±5.38	0.2834	0.1089
Subscapular skinfold (mm)	28.53±0.06	0.5787	**0.0001**	27.97±7.71	0.5718	**0.0012**
Total cholesterol (mmol/l)	4.48±1.30	0.2370	0.0560	4.10±0.71	–0.0512	0.7722
Testosterone (nmol/l)	1.33±0.67	0.0500	0.6870	4.83±4.03	0.0692	0.6955
DHEAS (µmol/l)	4.41±2.13	0.1672	0.1954	4.33±2.23	0.0501	0.7948
DHEA (nmol/l)	17.86±11.43	0.1667	0.2124	11.47±5.48	–0.1432	0.4570

*Correlation coefficients **r** (Pearson test) between fasting leptin concentrations, physical and metabolic characteristics are shown. **p**<0.05 values were considered significant.*

Leptin/testosterone ratio in obese boys was high in prepuberty. With advancing sexual development this ratio declines due to decreasing leptin and increasing testosterone values in puberty (Fig. 2). In obese girls leptin/testosterone ratio was high in prepuberty, but later decreased due to higher testosterone than leptin concentrations. At the end of puberty this ratio is high due to higher leptin concentrations than those of testosterone (Fig. 3).

Significant reductions in weight, body mass index and leptin were observed in all children after the intervention program (Table 2).

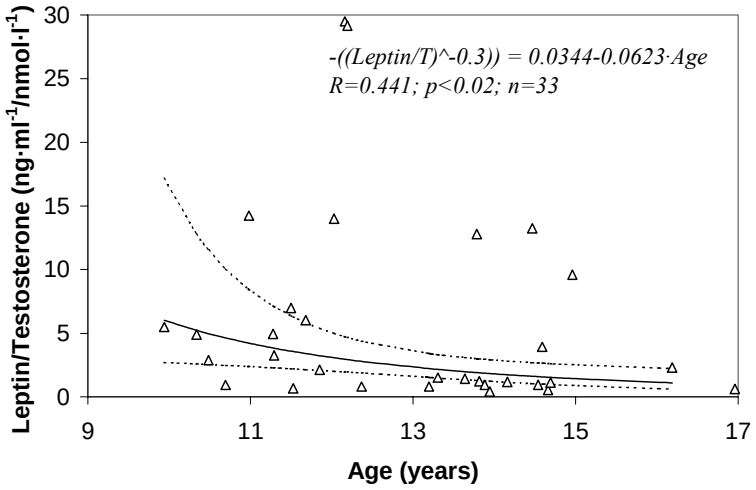

Fig. 2. *Leptin/testosterone ratio in relation to age in 33 obese boys. Full line represents regression line, whereas broken lines show 95 % confidence limits.*

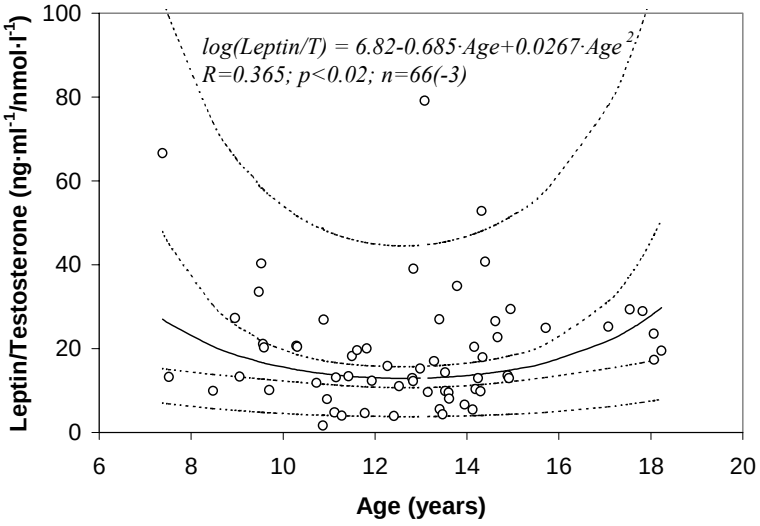

Fig. 3. *Leptin/testosterone ratio in relation to age in 66 obese girls. Full line represents regression line, whereas broken lines show 95 % and 90 % confidence limits.*

Discussion

In agreement with the study of Reiterer *et al.* (1999), leptin levels in obese children and adolescents correlated with BMI and were significantly reduced after the weight reduction program. The results of our study might be somewhat limited because we did not control leptin concentrations in lean children, nor did we investigate the obese children for a longer period of time.

Leptin levels reflect the amount of adipose tissue especially of subcutaneous fat (Lahlou *et al.* 1997). This is in agreement with our results that subscapular skinfold thickness (as an estimate of subcutaneous fat) was the strongest predictor of serum leptin concentrations in both genders. In young girls, the subcutaneous accumulation of body fat seems to be related in an almost parallel way to increasing levels of leptin during puberty, followed by an increase in FSH and later in LH and estradiol. At the same time, pulses in leptin secretion are higher in girls than in boys (Kiess *et al.* 1999). The differential evolution of leptin levels between boys and girls could be partly explained by the different evolution of body composition during puberty: whereas girls accumulate more fat mass, the increase in BMI in boys is mainly caused by an increase in muscle mass, especially during late puberty. Since leptin is mainly determined by fat mass, in particular by the amount of subcutaneous fat, this difference could partly explain the difference between boys and girls. The question remains open whether sex steroids play a role in this process.

Table 2. Serum leptin, weight, and BMI before and after a weight loss program in obese boys and girls.

	Obese girls (n = 66)		**Obese boys** (n = 33)		
	Before	**After**	**Before**	**After**	**p**
	Mean ± SD	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**	
Leptin (ng/ml)	20.79±9.61	13.50±8.65*	12.25±10.09	5.18±3.56*	<0.0001
Weight (kg)	73.24±17.20	65.52±15.73*	77.21±17.73	69.08±15.92*	n.s.
BMI (kg/m^2)	29.31±4.62	25.97±4.10*	30.46±4.54	27.00±4.09*	n.s.

*Data expressed as means ± SD, * significant difference from values before and after weight reduction (p<0.0001, ANOVA test).* ***p*** *shows for the difference between the sexes at beginning of the study (ANOVA test).*

The positive association of serum leptin with waist circumference and subscapular skinfold as well as their negative association with WHR in obese girls indicate a close relationship to android body fat distribution. It seems that testosterone could negatively influence leptin levels in obese girls with android obesity. This opinion is confirmed by the relationship of the leptin to testosterone ratio and age (Fig. 3.). A high androgenic activity in obese females is not only known to be a cause of menstrual irregularities and hirsutism, as observed in some of our patients, but may also be associated with metabolic disturbances. Many studies have demonstrated that women with hyperandrogenemia, regardless of its origin, have hyperinsulinemia (Wabitsch *et al.* 1995) which may be a marker of insulin resistance (Peiris *et al.* 1987). In addition, they have impaired glucose tolerance, lipid abnormalities and elevated blood pressure.

In girls, the leptin levels were significantly correlated with age, in contrast to boys. This is different from the observations in adults that show an inverse relationship between age and serum leptin concentrations in both men and women (Ostlund *et al.* 1996).

We observed sexual dimorphism in leptin concentrations. However, even after correcting for BMI the gender difference in leptin levels remains significant (p = 0.02). Sex steroid concentrations, body composition, and body fat distribution have been identified as important factors modulating the gender difference in leptin concentrations (Roemmich and Rogol 1999). Elevated leptin concentrations in obese girls seem to be closely connected with body weight and adipose tissue, perhaps to inform the brain about readiness of the body for reproductive functioning (Janečková 2001).

Several lines of evidence suggest that androgens mediate at least a portion of the pubertal reduction in serum leptin concentrations of boys. Androgens inhibit leptin secretion and leptin mRNA production in cultured adipocytes (Wabitsch *et al*. 1997). Testosterone alters the expression of proopiomelanocortin (POMC) mRNA in the neurons of the arcuate nucleus (Fodor *et al.* 2001). POMC neurons act as important mediators in the regulation of feeding behavior, insulin levels and, ultimately, body weight (Boston 2001). The proopiomelanocortin neurons in the arcuate nucleus have been established as leptin targets. Huszar *et al.* (1997) have shown that POMC-containing neurons in the arcuate nucleus are thought to function as feeding inhibitors. This effect could be a consequence of the action of testosterone.

Testosterone is a significant negative regulator of adipose tissue lipoprotein lipase (LPL) (Ramirez *et al.* 1997). Apart from a direct effect of testosterone at the gene level, it is possible that this suppression is mediated by indirect mechanisms of action such as an increase of ß-adrenoreceptors and a stimulation of lipolysis and fat free acids release (De Pergola 2000). It is known that obese men have low serum testosterone values (Bray 1997). In our group of obese boys, there were lower serum testosterone levels when compared with normal testosterone concentrations in lean boys (data not published). Our study did not show any correlation between leptin and testosterone in obese boys. This may be caused by low serum testosterone in obese boys, which could not effectively decrease leptin production. However, some effect of testosterone remains, which is supported by the relationship of leptin/testosterone ratio and age (Fig. 2). We demonstrated (Fig. 3) that a relationship between leptin and testosterone also exists in obese girls, although other studies have not shown any associations between leptin and testosterone in obese girls (Rosenbaum *et al.* 1996, Hassink *et al.* 1996, Garcia-Mayor *et al.* 1997). It is possible that higher testosterone

concentrations in obese girls than in lean girls (data not shown) are responsible for this discordance.

In our study, DHEA and DHEAS had no correlation with leptin concentrations in both genders. On the contrary, in the study of obese girls we found a weak inverse correlation of DHEAS associated with leptin (Wabitsch *et al.* 1997). De Pergola *et al.* (1996) demonstrated that plasma concentrations of DHEA were inversely related to total body fat and to subcutaneous adipose tissue area in premenopausal women. They proposed that the relationship might be the expression of a lower adrenal gland DHEA production and/or of a higher DHEA uptake in adipose tissue, as well as of a lipid mobilizing effect of DHEA. The results of the studies which examined the relationship between DHEA(S) and protective role in the incidence of cardiovascular disease in men are controversial (Poršová-Dutoit *et al.* 2000).

A number of large cohort studies have demonstrated an association between cholesterol and childhood obesity, and the tracking of cholesterol and other cardiac risk factors from childhood to adulthood (Bao *et al.* 1994, Raitakari *et al.* 1994). In our study, there was no significant correlation between total cholesterol and any anthropometric or biochemical parameters. These results correspond with the study of Byrnes *et al.* (1999).

Conclusions

We confirmed that leptin is a sensitive parameter of body composition and weight reduction in obese children. No relationship between leptin and cholesterol was found in either genders. Furthermore, there is no association between leptin and dehydroepiandrosterone or dehydroepiandrosterone sulphate. The relationship between leptin and testosterone is influenced by progress of puberty and is different in obese girls and boys.

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