SERUM LEPTIN LEVELS AND BODY COMPOSITION IN OBESE THAI CHILDREN

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Abstract. This study aimed to investigate the relationship between serum leptin concentrations and body composition among a sample of obese Thai children. A cross-sectional study was conducted in 158 schoolchildren, of whom 107 were obese and 51 normal weight; their mean age was 8.2 years. Body weight, height, waist circumference (WC), and subcutaneous skinfold thickness at 4 sites (triceps, biceps, subscapular, and supra-iliac) were measured. Total body fat (TBF) was determined by bioelectrical impedance analysis. Fasting blood samples were obtained to determine serum lipid profiles. The food intake of the children was estimated from interviews with the children and their mothers to elicit 24-hour food recall over 2 days. The results reveal subcutaneous fat skinfold, total body fat and waist circumference were significantly higher in obese than normal weight children ($p<0.001$). Serum leptin levels and lipid profile results, *ie* serum triglycerides (TG), serum total cholesterol (TC), low density lipoprotein cholesterol (LDL-C) and energy intake, were also significantly higher in the obese children than their normal-weight peers. Stepwise multiple regression analysis indicates that among boys, WC ($p<0.001$) and serum TG ($p=0.019$), and among girls, WC ($p<0.001$) and TBF ($p=0.030$), were significantly associated with leptin concentrations. No associations were found between leptin and energy intake in these children. A prospective study should investigate the influence of leptin levels on weight gain and subcutaneous adiposity, and the interrelationship between food intake and circulating leptin levels in children.

INTRODUCTION

Leptin, a product of the ob gene, is produced by adipocytes to regulate food intake and energy expenditure at the hypothalamic level (Inui,1999; Baile and Della-Fera, 2000) Evidence exists that most obese children have higher serum leptin concentrations than their normal weight peers, and that leptin concentrations positively correlate with accelerated gain in body weight (Moore *et al*, 2004; Nishimura *et al*, 2007). Leptin levels also exhibit significant changes during the process of puberty, and leptin concentrations tend to be higher among girls than boys (Demerath *et al*, 1999; Shalitin and Phillip, 2003). Furthermore, a few studies have shown that leptin concentrations are

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also predictive of significant increases in regional fat distribution in adults and children (Hu *et al*, 2001; Kettaneh *et al*, 2007). The correlation between blood leptin levels and cardiovascular risk factors was investigated in children of various ages, and revealed that higher plasma leptin concentrations correlated positively with higher blood lipid levels (Wu *et al*, 2001; Dubey *et al*, 2007), insulin levels and insulin resistance syndrome (Chu *et al*, 2000). However, little is known of the relationship between modifiable dietary factors and circulating leptin concentrations. One prospective study (Hakanen *et al*, 2004) did not find an association between leptin concentrations and energy or fat intake among schoolchildren, although there is evidence that food intake stimulates leptin secretion, perhaps through the direct action of insulin on adipocytes (Harris, 2000).

A few studies in Thai adults have shown higher leptin concentrations in the overweight and obese, compared with normal weight subjects; a positive association was found between serum leptin, body mass index (BMI), and waist circumference (Tungtronchitr *et al*, 2000, 2001), and soluble leptin receptors were significantly lower in overweight/obese adults (Popruk *et al*, 2005). Since little population-based information regarding circulating leptin in Thai schoolchildren is known, the aims of this study were: (1) to determine serum leptin concentrations in obese schoolchildren and normal weight schoolchildren; (2) to examine the relationship in children between leptin concentrations and body composition, lipid profile, and dietary factors.

MATERIALS AND METHODS

Subjects

This cross-sectional study was part of a previous study of malnutrition in 6 districts of Nakhon Pathom Province (Yamborisut *et al*, 2006). Purposive sampling was conducted among schoolchildren age 6-10 years old in grades 1-4 of 20 government primary schools in Nakhon Pathom Educational Service Areas 1 and 2 (Mueang, Sam Phran, Nakhon Chai Si, Phutthamonthon, Kamphang Saen, and Bang Len Districts), and 19 nearby government primary schools under the Education Department, Bangkok Metropolitan Administration (Bang Khae, Nong Khaem, and Taling Chan Districts). Obese and normal-weight children were screened at each school. Consent forms were obtained from the mothers of the children to conduct the study. The research design was approved by the Committee on Human Rights Related to Human Experimentation, Mahidol University.

Anthropometric measurements

The same researcher collected the children's anthropometric data throughout the study. The body weight of each subject was measured in light clothes using a beam balance scale (Soehnle-Waagen, Germany; capacity 5-150 kg) to the nearest 100 g. Height was measured with a stadiometer (Stanley-Mabo, France, No. 191) to the nearest 0.1 cm. The child's nutritional status was categorized using a weight-for-height Z-score (WHZ), Thai growth reference. An obese child was defined by a WHZ >+2 standard deviation (SD) above the median and a normal weight child was defined by a WHZ within ±1.5 SD of the median (Department of Health, 2000). Waist circumference (WC) was measured midway between the lowest rib and iliac crest, using non-stretchable tape. Four subcutaneous-skinfold sites were measured using a skinfold caliper (Holtain, Crymych, UK) to the nearest 0.2 mm. The triceps skinfold was measured at a mid-point of the upper right arm, between the scapular acromion process and the tip of the olecranon process. The biceps skinfold was measured at the anterior aspect of the forearm, at the same level as the triceps

skinfold. The subscapular skinfold site was measured 1 cm inferior to the inferior angle of the scapular and the suprailiac skinfold was measured immediately superior to the iliac crest. Measurements were taken in duplicate and average skinfold values estimated. The total body fat for each subject was determined by a bioelectrical impedance analyzer (Model BIA-101, RJL system, MI). The analyzer was calibrated before testing, using a 500-Ohm resistor provided by the manufacturer. Resistance and reactance were measured with a standard conduction current of 800 μA at 50 kHz. The subject lay supine with two electrodes placed on the dorsal surface of the right hand, and two on the dorsal surface of the right foot, as recommended for this system, and the total body fat was determined.

Biochemical assessment

Subjects were instructed to fast for at least 10-12 hours the previous night. Five milliliters of fasting blood were obtained from each subject via venipuncture before breakfast and separated for determination of serum lipids. Serum triglycerides (TG) and total cholesterol (TC) were determined by enzymatic kit (Ecoline^{R}; Diagnostica, Merck, Germany). TG were analyzed by glycerol-3-phosphate oxidase assay. TC was determined by enzymatic oxidation with cholesterol oxidase. Serum HDL-cholesterol (HDL-C) was measured by enzymatic kit (TRACE Liquid Stable HDL-Cholesterol^{R}, Thermo Trace, Australia). Analysis involved using selective inhibition of the non-HDL fraction with polyanion 4-aminoantipyrine, with subsequent reaction with cholesterol oxidase and cholesterol esterase. Serum LDL-cholesterol (LDL-C) was determined by the Friedewald formula (Friedewald *et al*, 1972). Serum leptin levels were measured by radioimmunoassay (Human leptin RIA kit, Cat.No. HL-81K, Linco Research, USA). The human leptin assay utilized ^{125}I-labeled human leptin and human leptin anti-serum to determine leptin levels (Ma *et al*, 1996). The detection limit of the assay was 0.5 ng/ml.

Nutrient intake

Information regarding food intake was obtained from the children and their mothers by interview. Nutrient intake was assessed using 24-hour recall for two days (one weekday and one weekend). Food models and some cooked food, including household measuring cups and spoons, were used to facilitate the estimation of food portion sizes during data collection.

Statistical analysis

Descriptive data of anthropometric parameters, serum leptin and lipid profiles, were expressed as mean ± standard deviation (SD). Data for the 24-hour food recalls, which were reported as measuring units, were converted to metric units; then, the nutrient intake of the children was computed using the INMUCAL program (Institute of Nutrition, 2005), which included a database of Thai foods. Overall data analysis was performed using the Statistical Package for Social Sciences (SPSS, version 13, Chicago, IL). Means for biochemical indices were compared between groups by Student's *t*-test. Multiple linear regression analysis was used to determine relationships between the serum leptin level, body composition, lipid profile results, and dietary factors.

RESULTS

The anthropometric and biochemical data of the children are shown in Table 1. The mean ages were 8.2 ± 1.1 years in the obese children, and 8.2 ± 0.9 years in the normal weight children. The mean body weight, WHZ, and WC in the obese children were significantly greater (p<0.001) than in the normal weight children. Obese children had significantly higher skinfold-thickness sums and percent total body fat, than the

Table 1
Anthropometric characteristics, serum leptin levels and lipid profiles in children, by nutritional status.

	Obese (*N*= 107)	Normal weight (*N*= 51)	*p*-value
Age (yrs)	8.2 ± 1.1[a]	8.2 ± 0.9	0.914
Weight (kg)	44.3 ± 8.1	24.2 ± 3.9	<0.001
Height (cm)	132.7 ± 7.1	125.1 ± 6.7	<0.001
WHZ	3.4 ± 1.1	-0.22 ± 0.7	<0.001
Waist circumference (cm)	79.4 ± 7.3	54.5 ± 4.4	<0.001
WHR	0.95 ± 0.05	0.86 ± 0.04	<0.001
Sum of 4 skinfolds (mm)	72.6 ± 12.7	26.5 ± 9.3	<0.001
% Total body fat	31.6 ± 4.2	14.9 ± 5.6	<0.001
Serum leptin (ng/ml)	23.7 ± 9.2	4.9 ± 2.7	<0.001
Serum TG (mg/dl)	110.2 ± 51.8	76.5 ± 31.7	<0.001
Serum total cholesterol (TC) (mg/dl)	210.8 ± 33.2	197.5 ± 23.8	<0.001
Serum LDL-C (mg/dl)	140.5 ± 33.4	129.9 ± 23.9	0.026
Serum HDL-C (mg/dl)	48.3 ± 8.9	52.2 ± 7.8	0.008
Energy intake (kcal/d)	1,412.9 ± 374	1,231.1 ± 313	0.003

[a] Mean ± SD
Significant mean difference between obese and normal weight children by Student's *t*-test, $p < 0.05$.
WHZ, weight-for-height Z score; WHR, waist-to-hip ratio; sum of 4 skinfolds, sum of biceps + triceps + subscapular +suprailiac skinfold thickness.

normal group. Serum leptin levels ($p<0.001$) and lipid levels; *ie*, TG ($p<0.001$), TC ($p<0.001$), LDL-C ($p=0.026$) and HDL-C ($p=0.008$) were significantly higher in obese than normal weight children. Mean energy intake was 1,412.9 ± 374 kcal/day in obese children, and 1,231.1 ± 313 kcal/day in normal weight children.

Differences were observed between boys and girls in each nutritional status group (Table 2). In the obese group, a significant difference in the mean was found between boys and girls in WHZ ($p=0.005$), WC ($p=0.002$), WHR ($p<0.001$), leptin levels ($p=0.042$), serum TG ($p=0.032$), and energy intake ($p=0.034$). In the normal weight group, significant mean differences in skinfold thickness sums ($p=0.002$), total body fat ($p=0.005$), and serum leptin levels ($p=0.002$), but not serum lipid levels or energy intake, were found between boys and girls.

Table 3 shows the relationship of leptin levels with anthropometric and biochemical parameters. Among boys, leptin levels positively correlated with WHZ ($r=0.763$, $p<0.001$), WC ($r=0.816$, $p<0.001$), WHR ($r=0.607$, $p<0.001$), skinfold-thickness sums ($r=0.799$, $p<0.001$), TBF ($r=0.776$, $p<0.001$) and the child's energy intake ($r=0.217$, $p=0.041$). Among girls, a significant positive correlation was also found between leptin levels and the same anthropometric parameters and energy intake as that in boys. Serum TG ($r=0.249$, $p=0.039$) in girls, were slightly positively correlated with leptin levels, whereas serum HDL-C levels in girls ($r = -0.256$, $p=0.033$) were negatively correlated with serum leptin levels.

Further analysis of associations between leptin levels and various parameters,

Table 2
Anthropometric characteristics, serum leptin levels and lipid profiles in all children, by gender.

Variable	Obese			Normal weight		
	Boys(*N*=66)	Girls(*N*=41)	*p*-value	Boys(*N*=23)	Girls(*N*=28)	*p*-value
Age (yrs)	8.1 ±1.2[a]	8.3 ±1.1	0.288	8.3 ± 0.9	8.1 ± 0.9	0.524
Weight (kg)	45.0 ± 8.5	43.0 ± 7.3	0.212	24.0 ± 3.9	24.5 ± 3.8	0.690
Height (cm)	133.0 ± 7.3	132.1 ± 6.8	0.509	125.1 ± 6.8	125.2 ± 6.7	0.957
WHZ	3.6 ± 1.2	3.0 ± 0.9	0.005	-0.35 ± 0.8	-0.11 ± 0.7	0.255
Waist circumference (cm)	81.1 ± 7.6	76.7 ± 5.9	0.002	54.1 ± 4.7	54.9 ± 4.2	0.528
WHR	0.97 ± 0.04	0.92 ± 0.04	<0.001	0.87 ± 0.04	0.85 ± 0.04	0.115
Sum of 4 skinfolds (mm)	72.9 ±13.8	72.0 + 10.8	0.710	22.2 + 8.1	30.1 + 8.8	0.002
% Total body fat	31.3 ± 4.6	32.2 ± 3.2	0.248	12.6 ± 4.7	16.9 ± 5.6	0.005
Serum leptin (ng/ml)	22.2 ± 9.4	25.9 ± 8.4	0.042	3.7 ± 2.4	6.0 ± 2.6	0.002
Serum TG (mg/dl)	101.8 ± 50.9	123.8 ± 51.1	0.032	71.8 ±18.7	80.4 ± 39.3	0.337
Serum total cholesterol (TC) (mg/dl)	209.2 ± 33.8	213.4 ± 32.5	0.531	192.4 ±25.6	201.7 ± 21.7	0.169
Serum LDL-C (mg/dl)	140.5 ±34.6	140.5 ± 31.9	0.997	126.5 ± 26.0	132.8 ± 22.0	0.350
Serum HDL-C (mg/dl)	48.4 ± 8.0	48.2 ± 10.4	0.904	51.6 ± 8.6	52.8 ± 7.3	0.600
Energy intake (kcal/d)	1,473.3 +372	1,315.9 + 362	0.034	1,271.7 + 275	1,197.6 + 343	0.406

[a] Mean ± SD
Significant mean difference between boys and girls in each group by Student's *t*-test, $p<0.05$.

by gender was conducted. Using stepwise multiple regression analysis, it was found (Table 4) that WC ($p< 0.001$) and serum TG ($p = 0.019$) were significant predictors of serum leptin level in boys, whereas WC ($p<0.001$) and TBF ($p=0.030$) were significant predictors of leptin levels in girls.

DISCUSSION

This study found total body fat, gender, waist circumference, and serum triglycerides, were associated with serum leptin levels in children. Obese children had significantly higher mean values for WHZ, waist circumference, body fat, skinfold thickness, and lipid levels (except for HDL-cholesterol) than their normal weight peers. Although a positive correlation between weight-for-height indices and serum leptin levels was seen among boys and girls, the results were inconsistent on multiple regression analysis. Previous studies confirmed a positive correlation between increased BMI and leptin levels in Thai children and adolescents (Aroonparkmonkol *et al*, 2005; Chaichanwatanakul *et al*, 2006). Nishimura *et al* (2007) also showed that serum leptin levels in 9-13 year-old schoolchildren were significantly positively correlated with percent overweight, irrespective of age or gender. Another study (Antunes *et al*, 2008) of overweight children with a mean age of 9.5 years indicated that BMI and gender were determinant factors of leptin levels. Leptin levels were also influenced by ethnic differences among children (Moore *et al*, 2004).

Table 2 shows the mean WHZ, WC, and WHR in girls in the obese group were significantly lower than in the boy group, while the total body fat in the girls tended to be greater than in the boys, although the difference was not significant due to small

Table 3
Pearson correlation between serum leptin levels and various parameters in children.

Parameters	Boys (*N*=89)		Girls (*N*=69)	
	r	*p*-value	*r*	*p*-value
WHZ	0.763	<0.001	0.821	<0.001
Waist circumference	0.816	<0.001	0.854	<0.001
WHR	0.607	<0.001	0.637	<0.001
Sum of 4 skinfolds	0.799	<0.001	0.869	<0.001
Total body fat	0.776	<0.001	0.830	<0.001
Serum TG	0.081	0.452	0.249	0.039
Serum total cholesterol (TC)	0.184	0.084	0.078	0.526
Serum LDL-C	0.165	0.123	0.076	0.537
Serum HDL-C	-0.024	0.823	-0.256	0.033
Child's energy intake	0.217	0.041	0.279	0.020

Table 4
Multiple linear regression analysis with serum leptin levels as a dependent variable in children.

Independent variables	Unstandardized coefficients (β)	SE (β)	*p*-value	Adjusted model R^2
Boys (*N*=89)				
Constant	-32.173	3.826	<0.001	0.680
Waist circumference	0.717	0.052	<0.001	
Serum triglycerides	-0.037	0.015	0.019	
Girls (*N*=69)				
Constant	-31.510	5.555	<0.001	0.740
Waist circumference	0.560	0.143	<0.001	
Total body fat	0.438	0.197	0.030	

sample size. Weight-for-height indices, which are composite measures of fat mass and muscle mass, have been seen as inconsistent indicators of the amount of leptin-associated adipose tissue. In the normal weight group, despite similarities in weight status, girls had significantly higher body fat and leptin levels than boys. Garcia-Mayor *et al* (1997) showed, in normal weight children aged 5-15 years, that leptin levels in boys were always lower than in girls, although they increased with age. The lower leptin levels in boys may be partly explained by the suppressive effects of androgen (Blum *et al*, 1997). This sexual dimorphism could be explained by evidence that leptin secretion from adipose tissues is 2- to 3-fold higher for females than males (Licino *et al*, 1998). Another study showed that females have higher total serum leptin levels, but lower leptin-binding protein levels, than males, indicating higher free leptin levels (McComway *et al*, 2000). Furthermore, another study revealed the pubertal stage

could affect changes in leptin levels, and that female pubertal children had higher leptin levels than male children, even after correcting for body weight (Hassink *et al*, 1996). Serum leptin levels rise in young girls at the age of 7 years and continue to rise as they progress through puberty until about age 15 years (Blum *et al*, 1997; Garcia-Mayor *et al*, 1997; Ahmed *et al*, 1999). In boys, leptin levels seem to rise transiently and then decline after Tanner stage 2. The limitation of our study was that the pubertal stage was not identified in some of the older children; we did not examine the effects of puberty on leptin levels.

Table 4 shows that waist circumference and serum TG explain 68% of the variation in circulating leptin levels in boys, whereas waist circumference and total body fat predict 74% of the variation of leptin levels in girls. Because leptin is synthesized by adipocytes, its concentration correlates with body fat. Previous studies in both normal weight and obese children revealed a positive correlation between leptin levels and total body fat (Dencker *et al*, 2006; Fleisch *et al*, 2007), or between leptin levels and the sum of subcutaneous fat (Roemmich *et al*, 1998; Sudi *et al*, 2000). However, no associations were found between leptin levels and skinfold thickness sums in our study. These findings should be investigated further, since it may be hypothesized that leptin levels may be dependent on some specific sites of subcutaneous adiposity, such as the abdominal region, rather than other sites. In our study, waist circumferences of both genders were found to be positively correlated with leptin levels similar to some previous studies (Kelishadi *et al*, 2006; Dubey *et al*, 2007). This suggests that changes in waist circumference may be one potential indicator of serum leptin levels in humans.

The positive correlation between serum leptin levels and triglycerides has been investigated in many studies (Chu *et al*, 2000; Steinberger *et al*, 2003). This relationship is complex and may be explained by obesity in children. Obesity is associated with leptin resistance, which may be caused by a defect in leptin receptors (Popruk *et al*, 2008). There is evidence that elevated triglyceride levels could inhibit leptin transport across the blood-brain barrier, as assessed by an *in vivo* study (Banks *et al*, 2004).

It has been reported that leptin plays a key role in regulating food intake. The lack of the inhibiting effect of leptin as shown by higher energy intake in obese children who had higher serum leptin levels in our study suggests the possibility of the occurrence of leptin resistance in obese children, as reported by previous human studies (Kolaczynski *et al*, 1996; Klok *et al*, 2007). However, this cross-sectional study did not permit a cause-effect relationship to be established, so that a prospective study is needed to explore the interrelationship between over-eating and changes in circulating leptin levels.

In conclusion, this study shows that serum leptin levels in schoolchildren were significantly associated with waist circumference, serum triglyceride levels and body fat and that obesity status and gender were influencing factors.. The relationship of leptin levels to specific sites of subcutaneous adiposity, such as abdominal fat, should be further investigated. The influence of food intake on changes in circulating leptin should also be explored in a long-term prospective study.

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