LEPTIN CONCENTRATION IN RELATION TO BODY MASS INDEX (BMI) AND HEMATOLOGICAL MEASUREMENTS IN THAI OBESE AND OVERWEIGHT SUBJECTS

Rungsunn Tungtrongchitr[1], Praneet Pongpaew[1], Benjaluck Phonrat[2], Siriwan Tribunyatkul[1], Duangkamol Viroonudomphol[1], Venus Supawan[1], Pornrutsami Jintaridhi[1], Ariya Lertchavanakul[3], Niyomsri Vudhivai[1] and Frank Peter Schelp[4]

[1]Department of Tropical Nutrition and Food Science, Faculty of Tropical Medicine, Mahidol University, 420/6 Rajvithi Road, Rajthevee, Bangkok 10400, Thailand; [2]Department of Tropical Medicine, Faculty of Tropical Medicine, Mahidol University, 420/6 Rajvithi Road, Rajthevee, Bangkok 10400, Thailand; [3]Out-patient Department, General Practice Section, Rajvithi Hospital, Rajthevee, Bangkok 10400, Thailand; [4]Center for Humanity and Health Sciences, Free University Burlin and Humbolt University, Burlin, Germany

Abstract. The weight, height and body mass index (BMI), including waist/hip ratio, serum leptin and hematological parameters of 48 male and 166 female overweight (BMI ≥ 25.00) Thai volunteers who came for a physical check-up at the Out-patient Department, General Practice Section, Rajvithi Hospital, Bangkok during the period March-October 1998, were investigated. There were statistically significantly higher levels of serum leptin, mean corpuscular mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular volume (MCV) in the overweight than in the control subjects. The median serum leptin concentration in overweight subjects was 19.6 (2.0-60.0 ng/ml) compared with 9.0 (range 1.0-30.0 ng/ml) in the control subjects ($p < 0.001$). The medians of leptin in overweight and obese males were significantly higher than those of overweight and obese females. 66.7% (32 out of 48) of overweight and obese males were found to have elevated leptin levels, while 87.3% (145 out of 166) were found in overweight and obese females. Anemia was found in 18.7% of female overweight and obese subjects, using hemoglobin as an indicator. Significant associations were found between weight, height, BMI, waist, hip, waist/hip ratio, hemoglobin, hematocrit, and serum leptin in both male and female overweight subjects. A negative correlation was found between serum leptin and hemoglobin, and hematocrit in both overweight and obese subjects.

INTRODUCTION

Leptin, the *ob* gene product secreted by adipocytes, decreases food intake while it increases energy expenditure and functions as an important signal for the regulation of body weight (Frederich *et al*, 1995; Halaas *et al*, 1995; Pelleymounter *et al*, 1995). Leptin has been shown to correlate with fat mass and body weight in normal and obese patients (Considine *et al*, 1996; Maffei *et al*, 1995; Pelleymounter *et al*, 1995; Stephens *et al*, 1995; Zhang *et al*, 1994). In mice, mutations in the *ob* gene that result in a lack of circulating leptin cause obesity. The administration of recombinant leptin causes weight loss in these mice. It is thought that leptin is integral in the feedback loop from adipose stores to the satiety centers in the hypothalamus (Mercer *et al*, 1996) causing a decrease in appetite and increase in energy expenditure. An increase in the expression of the *ob* gene has been found in the adipocytes of obese adults (Considine *et al*, 1995; 1996; Hamilton *et al*, 1995; Lönnqvuist *et al*, 1995; Zhang *et al*, 1994). Leptin has been detected in the serum of obese and normal-weight adults, with obese adults having significantly higher serum leptin concentrations than do normal-weight adults. Elevations of serum leptin concentrations were most closely correlated with the percentage of body fat (Mercer *et al*, 1996). Recent studies showed that leptin plus erythropoietin acted synergistically to increase erythroid development *in vitro* (Bennett *et al*, 1996; Mikhail *et al*, 1997).

In this study we investigated whether leptin protein was correlated with hematological parameters and body mass index in healthy overweight and obese Thais compared with normal subjects. This would both establish baseline values for these parameters and allow us to investigate the changes in leptin in obese Thais.

Correspondence: Assoc Prof Rungsunn Tungtrongchitr, Department of Tropical Nutrition and Food Science, Faculty of Tropical Medicine, Mahidol University, 420/6 Rajvithi Road, Rajthevee, Bangkok 10400, Thailand.
Tel: 66-2-2485748; Fax: 66-2-2485748

MATERIALS AND METHODS

Study population

Forty-eight male and 166 female overweight and obese Thai volunteers, including 26 male and 81 female normal subjects, comprised the study population. Thai volunteers who attended the Outpatient Department, General Practice Section, Rajvithi Hospital, Bangkok, for a physical check-up during the period March-October 1998, were investigated for this study. The age, marital status, place of origin, drinking and smoking habits were assessed by standardized questionnaires. The same medical doctor conducted physical examinations throughout the study.

Analytical methods

The nutritional status of all subjects under investigation was assessed by means of anthropometric measurements. The body weight of each individual dressed in light clothing was measured using a carefully calibrated beam balance (Detecto®, Detecto scale manufacturing, USA). Height measurements were taken using a vertical-measuring rod. BMI or Quetelet Index was conventionally calculated as weight in kg/(height in meters)^2. The classifications of BMI employed were those used by the WHO Expert Committee 1995 (WHO, 1995), overweight grade I : BMI=25.00-29.99; grade II (obese) : BMI=30.00-39.99; grade III (obese) : BMI≥40 kg/m^2. Waist and hip circumferences were also measured in order to calculate waist/hip ratio (normal value for female <0.77, male <0.90) (Dowling and Pi-Sunyer, 1993; Seidell *et al*, 1994).

From the subjects under study, about 10 ml of venous blood was taken in the morning after fasting overnight. Heparinized blood was used to determine hematological status. The blood samples were stored at 2-5ºC for not more than 24 hours prior to laboratory investigations. Hemoglobin concentration and hematocrit values were determined. A serum aliquot was stored frozen at -20ºC for serum leptin.

Laboratory techniques

Hemoglobin concentrations in whole blood were determined using the modified cyanomethemoglobin method (International Committee, 1978). Hematocrit values were measured by a micromethod using calibrated heparinized capillary tubes. After filling the capillaries with blood, they were centrifuged for 5 minutes at 14,000*g* in an IEC MB microhematocrit centrifuge. Hematocrit values were measured using a micro-hematocrit reader (Hawksley, England). Red cell count was measured on an STKR cell counter (Coultronics, Margency, France). Mean corpuscular hemoglobin concentrations (MCHC) and mean corpuscular volume (MCV) were also calculated by the formula as following:

$$\text{MCHC (g/dl)} = \frac{\text{Hemoglobin (g/dl) x 100}}{\text{Hematocrit (\%)}}$$

$$\text{MCV} = \frac{\text{Hematocrit x 10}}{\text{Red cell count (millions/mm}^3\text{)}}$$

Serum leptin was assessed using a commercially available radioimmunoassay from Linco Research Inc utilizing ^{125}I-labeled human leptin and a human leptin antiserum (Ma *et al*, 1996).

Statistical analysis

The data were analyzed using standard statistical methods provided by the Minitab statistical computer program (Ryan *et al*, 1985). The median, range and 95% confidence interval (CI) were calculated. The Mann Whitney U-Wilcoxon Rank Sum W test (two tailed) was used for calculating statistical differences between groups.

RESULTS

Median, range and 95% confidence interval (CI) of age, anthropometric variables, serum leptin concentration and hematological parameters in overweight and control subjects are shown in Table 1. All of the anthropometric variables, except the height of the overweight group, were significantly higher than those of normal subjects. There were statistically significantly higher levels of serum leptin, MCHC and MCV in the overweight than in the control subjects. The median serum leptin concentration in overweight subjects was 19.6 (2.0-60.0 ng/ml) compared with 9.0 (range 1.0-30.0 ng/ml) in the control subjects ($p < 0.001$). The medians of leptin in overweight and obese males were significantly higher than those of overweight and obese females (Table 2).

Using the cut-off point of leptin concentration suggested by Ma *et al*, (1996), 66.7% (32 out of 48) of overweight and obese males were found

Table 1
Median, ranges and 95% confidence interval (CI) of age, anthropometric variable, leptin and hematological parameters in overweight and control subjects.

Parameter	Total				p-value[a]
	Overweight (N=214)		Control (N=107)		
	Median (range)	95%CI	Median (range)	95%CI	
Age (yrs)	38 (18-58)	37-40	36.5 (18-55)	33-40	0.233
Weight (kg)	76.7 (54.0-129.2)	75.0-79.9	54.3 (42.5-78.0)	52.7-56.0	0.000
Height (m)	1.56 (1.45-1.84)	1.55-1.57	1.59 (1.43-1.85)	1.57-1.60	0.088
BMI (kg/m^2)	31.01 (25.19-53.28)	30.48-31.64	21.85 (18.18-24.82)	21.01-22.35	0.000
Waist (cm)	91.0 (66.5-127.0)	89.6-93.0	72.5 (60.0-98.0)	71.4-74.0	0.000
Hip (cm)	108.0 (86.5-151.5)	106.0-110.0	92.0 (82.0-102.0)	92.0-94.0	0.000
Waist/Hip ratio	0.84 (0.67-1.01)	0.83-0.85	0.78 (0.65-0.93)	0.77-0.80	0.000
Leptin (ng/ml)	19.6 (2.0-60.0)	18.0-21.9	9.0 (1.0-30.0)	8.3-10.2	0.000
Hemoglobin (g/dl)	13.4 (8.3-17.1)	13.2-13.5	13.4 (9.2-17.5)	13.1-13.5	0.570
Hematocrit (%)	40.1 (26.6-49.7)	39.5-41.0	40.6 (27.8-54.0)	39.9-41.0	0.978
MCHC	33.8 (23.8-35.9)	33.6-34.1	33.6 (31.5-36.4)	33.3-33.7	0.003
MCV	82.2 (59.2-91.6)	81.4-83.3	84.5 (57.9-96.2)	83.2-86.4	0.001

BMI = body mass index.
MCHC = mean corpuscular hemoglobin concentration.
MCV = mean corpuscular volume.
[a]Mann-Whitney U-Wilcoxon Rank Sum W test (Two-tailed).

to have elevated leptin levels while 87.3% (145 out of 166) were found in overweight and obese females (Table 3). Anemia was present in about 18.7% of female overweight and obese subjects, using hemoglobin as an indicator. However, a higher percentage of anemia was found when MCHC was used as an indicator of anemia.

Tables 4 and 5 show the correlation coefficients between various parameters in male and female overweight subjects, respectively. Significant associations were found between weight, height, BMI, waist, hip, waist/hip ratio, hemoglobin, hematocrit, and serum leptin in both male and female overweight subjects. A negative correlation was found between serum leptin and hemoglobin as well as hematocrit in both overweight and obese subjects. There was a significant positive correlation between height, waist, hip, waist/hip ratio with hemoglobin and hematocrit, in both male and female overweight subjects.

DISCUSSION

In our study, higher leptin concentration was also found in Thai overweight and obese subjects

Table 2
Medians, ranges and 95%(CI) of age, anthropometric variable, leptin and hematological parameters in overweight and control subjects between male and female.

Parameter	Male				p-value	Female				p-value[a]
	Overweight (N=48)		Control (N=26)			Overweight (N=166)		Control (N=81)		
	Median (range)	95%CI	Median (range)	95%CI		Median (range)	95%CI	Median (range)	95%CI	
Age (yrs)	40.5 (18.0-55.0)	38.8-45.2	35.5 (19.0-54.0)	30.0-42.0	0.069	37.0 (18.0-58.0)	35.0-39.1	37.0 (18.0-55.0)	33.0-40.0	0.782
Weight (kg)	85.7 (62.4-114.8)	81.0-91.5	61.8 (50.7-78.0)	58.9-64.7	0.000	74.8 (54.0-129.2)	72.9-76.7	52.5 (42.5-67.0)	51.4-54.2	0.000
Height (m)	1.69 (1.50-1.84)	1.67-1.72	1.65 (1.57-1.85)	1.65-1.71	0.522	1.55 (1.45-1.69)	1.54-1.56	1.56 (1.43-1.69)	1.54-1.59	0.054
BMI (kg/m^2)	30.58 (25.35-38.65)	29.18-31.93	21.74 (18.68-24.61)	20.61-23.23	0.000	31.13 (25.19-53.28)	30.50-31.91	21.85 (18.18-24.82)	20.94-22.31	0.000
Waist (cm)	98.5 (66.5-122.0)	93.0-103.0	80.5 (64.0-89.0)	74.7-83.7	0.000	89.8 (71.0-127.0)	86.0-91.0	71.0 (60.0-98.0)	70.0-72.4	0.000
Hip (cm)	98.5 (89.5-129.0)	103.4-110.0	94.0 (87.0-102.0)	91.0-95.4	0.000	108.0 (86.5-151.5)	106.0-110.0	92.0 (82.0-111.0)	91.6-93.2	0.000
W/H ratio	0.92 (0.67-1.01)	0.91-0.94	0.87 (0.72-0.93)	0.82-0.88	0.000	0.83 (0.70-0.99)	0.81-0.84	0.77 (0.65-0.88)	0.76-0.78	0.000
Leptin (ng/ml)	7.8 (2.0-30.0)	6.1-9.8	3.5 (1.0-9.0)	2.5-5.0	0.000	22.6 (8.0-60.0)	20.2-25.0	10.5 (2.6-30.0)	9.7-11.5	0.000
Hemoglobin (g/dl)	14.9 (12.1-17.1)	14.4-15.1	15.1 (12.5-17.5)	14.0-15.3	0.928	13.0 (8.3-15.9)	12.9-13.3	13.0 (9.2-14.6)	12.6-13.3	0.368
Hematocrit (%)	45.1 (35.4-49.7)	43.9-45.6	45.5 (38.8-54.0)	43.0-46.0	0.713	39.3 (26.6-48.0)	38.6-40.0	39.4 (27.8-44.3)	38.1-40.2	0.738
MCHC	33.7 (30.9-35.3)	33.5-34.1	33.2 (31.7-34.6)	32.5-33.7	0.017	33.9 (23.8-35.9)	33.6-34.1	33.6 (31.5-36.4)	33.4-33.8	0.033
MCV	83.2 (64.1-90.3)	81.0-84.6	85.2 (60.3-91.5)	77.4-88.3	0.236	82.0 (59.2-91.6)	80.3-83.1	84.5 (57.9-96.2)	83.2-86.5	0.001

BMI = body mass index.
MCHC = mean corpuscular hemoglobin concentration.
MCV = mean corpuscular volume.
[a]Mann-Whitney U-Wilcoxon Rank Sum W test (Two-tailed).

Table 3
Number and percentage of individuals with overweight, abnormal leptin, anemia in overweight subjects.

Parameter	Male		Female		Total	
	N/Total	%	N/Total	%	N/Total	%
Grading of overweight by BMI (kg/m^2)			62/166	37.3	82/214	38.3
Grade I (BMI=25.00-29.99)	20/48	41.7	93/166	55.7	121/214	56.5
Grade II (BMI=30.00-39.99)	28/48	58.3	11/166	6.6	11/214	5.2
Grade III (BMI ≥ 40.00)	-	0.0			181/214	84.2
Waist/Hip ratio						
Male ≥ 0.90	36/48	75.0	145/166	87.3		
Female ≥ 0.77					181/214	84.6
Leptin						
Male >5.6 ng/ml	32/48	66.7	149/166	89.8		
Female >10.8 ng/ml						
Anemia					35/214	16.4
Male Hb <13.0 g/dl	4/48	8.3	31/166	18.7		
Female Hb <12.0 g/dl					32/214	14.9
Male Hct <40.0%	4/48	8.3	28/166	16.9		
Female Hct <36.0%			29/166	17.5	38/214	17.7
Both sexes MCHC <33.0 %	9/48	18.8				

Table 4
Correlation coefficients of age, anthropometric variables, leptin and hematological parameters in both overweight males and females (BMI ≥ 25.0 kg/m^2).

Parameter	Leptin	Hemoglobin	Hematocrit	MCHC	MCV
Age	-0.291[b]	0.073	0.069	0.085	0.032
Weight	0.355[b]	0.088	0.116	-0.179[b]	-0.029
Height	-0.322[b]	0.289[b]	0.298[b]	-0.077	-0.021
BMI	0.618[b]	-0.078	-0.052	-0.154[a]	-0.015
Waist	0.275[b]	0.611[b]	0.167[a]	-0.236[b]	-0.118
Hip	0.576[b]	-0.086	-0.057	-0.195[b]	-0.085
Waist/Hip ratio	-0.170[a]	0.276[b]	0.300[b]	-0.146[a]	-0.081
Leptin	1.000	-0.259[b]	-0.237[b]	-0.084	-0.018
Hemoglobin	-0.259[b]	1.000	0.973[b]	0.209[b]	0.407[b]
Hematocrit	-0.237[b]	0.973[b]	1.000	0.073	0.302[b]
MCHC	-0.084	0.209[b]	0.073	1.000	0.624[b]
MCV	-0.118	0.407[b]	0.302[b]	0.624[b]	1.000

Significant difference: [a]$p<0.05$, [b]$p<0.01$.

(BMI ≥ 25.0 kg/m^2) when compared with normal control subjects. The medians of leptin in overweight and obese males were significantly higher than those of overweight and obese females. Leptin was identified in 1994 as the product of a gene that is defective in an obese strain of mice, *ob/ob* (Zhang *et al*, 1994). The obese gene encodes leptin, a 16-kDa peptide, 167 amino acid is secreted by fat cells (adipocytes). Treatment of *ob/ob* mice with leptin reversed all the manifestations of the *ob/ob* phenotype and also caused weight loss in wild-type mice (Campfield *et al*, 1995; Halaas *et al*, 1995; Stephens *et al*, 1995). Leptin was found in high concentrations in obese children and was highly correlated with arm fat area and BMI (Hassink et al, 1996). Leptin acts to decrease weight and adipose tissue mass through decreases in appetite and food intake (Campfield *et al*, 1995; Halaas *et al*, 1995; Pelleymounter *et al*, 1995). Although a high leptin concentration was found to

Table 5
Correlation coefficients of age, anthropometric variables, leptin and hematological parameters in both overweight males and females (BMI ≥ 30.0 kg/m^2).

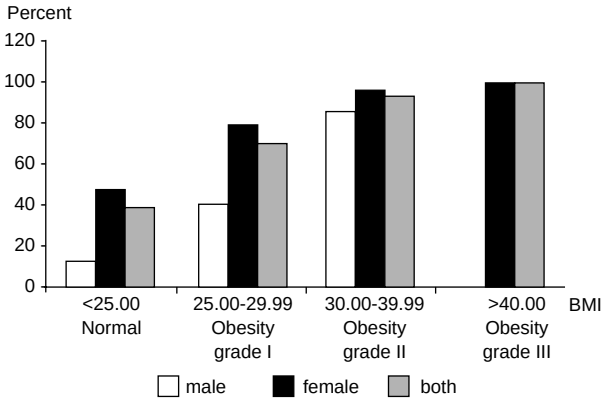
Parameter	Leptin	Hemoglobin	Hematocrit	MCHC	MCV
Age	-0.164	0.076	0.083	0.040	0.026
Weight	0.227[b]	0.007	0.023	-0.098	-0.166
Height	-0.349[b]	0.248[b]	0.261[b]	-0.061	-0.048
BMI	0.550[b]	-0.184[a]	-0.178[a]	-0.061	-0.150
Waist	0.189[a]	0.047	0.061	-0.122	-0.199[a]
Hip	0.557[b]	-0.177[a]	-0.159	-0.126	-0.219[a]
Waist/Hip ratio	-0.231[b]	0.220[a]	0.223[a]	-0.049	-0.069
Leptin	1.000	-0.350[b]	-0.346[b]	-0.064	-0.117
Hemoglobin	-0.350[b]	1.000	0.973[b]	0.345[b]	0.498[b]
Hematocrit	-0.346[b]	0.973[b]	1.000	0.225[b]	0.391[b]
MCHC	-0.064	0.345[b]	0.225[b]	1.000	0.663[b]
MCV	-0.117	0.498[b]	0.391[b]	0.663[b]	1.000

Significant difference: [a]$p<0.05$, [b]$p<0.01$.

positively correlate with the degree of obesity in humans, a high level of this hormone does not function to decrease weight as in the mouse experiments. The reason for this may be the presence of abnormal leptin protein and leptin receptor protein. The mutation occurring on the *ob* gene of the obese person resulted in the malfunction of leptin protein. DNA of the obese persons has been studied for the presence of mutations in the human *ob* gene, but no mutations were found in the *ob* genes that could be responsible for the obesity of these obese persons (Maffei *et al*, 1996; Niki *et al*, 1996; Schwartz *et al*, 1997). The mutation of the leptin gene has been demonstrated in Pakistani and Turkish obese subjects (Montague *et al*, 1997; Strobel *et al*, 1998) but this is not secreted in serum (Strobel *et al*, 1998). More recently, a mutation in the human leptin receptor was demonstrated (Clement *et al*, 1998; Gotoda *et al*, 1997). This mutation resulted in the synthesis of a truncated receptor lacking both the transmembrane and the intracellular domain. It blocks binding of leptin to the membrane-bound receptor cell in the hypothalamus (Liu *et al*, 1997) and led to a hypothesis of a resistance to the action of leptin at the level of the hypothalamus (Rohner Jeanrenaud and Jeanrenaud, 1996; Rosenbaum and Leibel, 1988) causing increased appetite and decreased energy expenditure despite adequate leptin production by adipocytes. More recently, it was found that blood-brain barrier transportation has a threshold level of serum leptin (about 25-30 nanogram/ml) above which increases in serum levels are not translated into proportional increases in cerebrospinal or brain leptin levels; this means that it may result in an apparent leptin resistance and obesity (Caro *et al*, 1996). Thai obese subjects in this study might be leptin resistant; however, the causes of resistance are unknown, both in the case of abnormal leptin protein production or abnormality of the leptin receptor gene. Further study may be required.

Our results also shows that women seem to have higher leptin levels than men, even when the fat mass or BMI was adjusted (Table 2 and Fig 1). This might be because of their different body fat distribution or the inducing effects of estrogen progesterone combined with the suppressive effect of androgens on leptin (Hickey *et al*, 1997; Lönnqvuist *et al*, 1995; Ostlund *et al*, 1996; Rosenbaum *et al*, 1996; Saad *et al*, 1997; Schrauwen *et al*, 1997; Shimizu *et al*, 1997).

A negative correlation between hemoglobin and serum leptin was found in male and female overweight and obese subjects (Tables 4, 5). This result agreed with the study of Toko *et al*, (1999) who reported a negative correlation between the levels of leptin and those of hemoglobin in Japanese men (Toko *et al*, 1999). Erythropoiesis is thought to be regulated by erythropoietin, which in adults, is produced mainly in the kidneys, in response to hypoxia (Wang and Semenza, 1996). Leptin production occurs mainly in adipocytes, but there has been no report showing that adipocytes have a sensor for hypoxia. It is interesting that



*Value indicating elevated serum leptin level are; for male >5.6 ng/ml, female >10.8 ng/ml (Ma *et al*, 1996).

Fig 1–Percentage of elevated serum leptin* in different grade of obesity.

bone marrow contains many adipocytes, the role of which is not clear. With the epidemiologic findings (Bennett *et al*, 1996) and the studies performed *in vitro* (Bennett *et al*, 1996; Mikhail *et al*, 1997) it is suggested that leptin may play some role in hematopoiesis in humans. Further research into the pathophysiology of leptin and hematopoiesis might be needed for more information on this relationship.

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