Serum Leptin Levels and Their Association with Several Factors Related to Arteriosclerosis among Medical Students in Japan

Motonobu MIYAZAKI*¹, Akihiko KAETSU*², Yoshito MOMOSE*¹ and Hiroshi UNE*¹

*¹Department of Hygiene and Preventive Medicine, School of Medicine, Fukuoka University, Fukuoka

*²Department of Public Health, School of Medicine, Fukuoka University, Fukuoka

Abstract

The association between serum leptin levels and several factors related to arteriosclerosis were studied in subjects who were Japanese medical students taking no medications. The group was comprised of 75 males and 35 females. The age distribution in males was 21.4 to 29.8 years (median age, 24.0 years) and in females was 21.3 to 29.9 years (median age, 22.9 years). Statistical analyses were performed using the Wilcoxon rank sum test and Spearman correlation. Median levels of serum leptin were 3.3 ng/ml in males and 7.6 ng/ml in females. The largest correlation observed was with percent body fat irrespective of sex [males; $r=0.775$, $p<0.001$, females; $r=0.553$, $p<0.001$]. However, body mass index (BMI) was similarly well correlated with serum leptin [$r=0.631$, $p<0.001$] in males but not in females [$r=0.305$, $p=0.075$]. A negative correlation was observed between high density lipoprotein cholesterol (HDL-C) and leptin in both sexes [males; $r=-0.298$, $p<0.01$, females; $r=-0.363$, $p<0.05$], respectively. Percent body fat, BMI, diastolic blood pressure, apolipoprotein B, and HDL-C were the most significant factors in males. Percentage of body fat and HDL-C were the most important factors in females.

Key words: serum leptin, medical students, sex difference, percent body fat, HDL-C

Introduction

Obesity is among the most common health problems in industrial societies. Obesity is defined by an excess of adipose tissue which is a health risk. Leptin is secreted from adipose tissue (1) and appears to be a component of the regulatory loop linking fat mass to food intake and energy expenditure (2). Leptin may provide an afferent signal in a feedback loop that regulates body mass fat and hence may play a role in the pathogenesis of obesity (3). Previous studies have reported a significant correlation between leptin concentrations and obese humans (4,5). However, only a few epidemiological studies on leptin among normal young adults have been previously reported (6,7). We measured serum concentrations of serum leptin among a twenty-year age group of subjects and discussed the relationships between serum leptin and arteriosclerosis related factors of subjects.

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Reprint requests to: Motonobu MIYAZAKI,
Department of Hygiene and Preventive Medicine, School of Medicine,
Fukuoka University, 7-45-1 Nanakuma, Jonan-ku, Fukuoka City, Fukuoka
814-0180, Japan.

TEL : +81(92)801-1011 ext 3301

FAX : +81(92)863-8892

Materials and Methods

Subjects

Serum leptin levels was measured in one hundred and ten medical students in the 4th year. Subjects aged from 21 to 29 years were in good health and taking no medications. The group included 75 males and 35 females. All subjects provided informed consent before participation. Blood samples were obtained in June, 1997. Height and weight were measured while the subjects were fasting and wearing only their undergarments. Weight was recorded to the nearest 0.1 Kg and height to the nearest 0.1 cm. The percentage of body fat was measured automatically by the Tanita Model TBF-102 (8,9). Body mass index (BMI) was calculated as weight (in kilograms) divided by height (in meters) squared. Blood pressure was measured by the use of a sphygmomanometer (10).

Procedure

Blood samples were drawn into siliconized disposable plastic tubes containing 1 mg/ml EDTA from a peripheral vein between 8:00 and 8:30 in the morning after an overnight fast and

Table 1 Descriptive characteristics

Variable	Median value (range)	p value
	Male (n=75)	Female (n=35)
Age (year)	24.0 (21.4- 29.8)	22.9 (21.3- 29.9)
Height (cm)	171.8 (160.0-186.0)	159.7 (150.1-159.7)
Weight (kg)	67.1 (44.6- 99.3)	50.2 (41.8- 61.5)
BMI (kg/m ²)	22.7 (17.0- 34.0)	19.7 (17.1- 21.3)
Body fat (%)	20.9 (10.5- 38.7)	23.6 (17.6- 30.9)
SBP (mmHg)	120.6 (88.0-163.0)	102.4 (86.0-122.0)
DBP (mmHg)	74.8 (49.0- 97.0)	67.3 (43.0- 87.0)
TC (mg/dl)	187.8 (115.0-333.0)	177.0 (129.0-249.0)
HDL-C (mg/dl)	56.8 (33.0- 98.0)	68.0 (34.0- 92.0)
Apo A-I (mg/dl)	147.5 (96.0-235.0)	145.8 (82.0-194.0)
Apo A-II (mg/dl)	37.5 (26.0- 74.0)	31.1 (24.0- 41.0)
Apo B (mg/dl)	89.7 (37.0-208.0)	65.9 (38.0-108.0)
Leptin (ng/ml)	3.3 (1.2- 15.9)	7.6 (1.8- 23.4)

*, **, p<0.01, p<0.001 by Wilcoxon rank sum test

BMI; Body mass index. SBP, DBP; Systolic and diastolic blood pressure

Table 2 Spearman correlation of serum leptin concentrations with selected variables

Variable	Male (n=75)		Female (n=35)	
	r	p value	r	p value
Age	0.146	0.210	0.132	0.450
BMI	0.631	<0.001	0.305	0.075
Body fat	0.775	<0.001	0.553	<0.001
SBP	0.339	<0.01	0.011	0.951
DBP	0.513	<0.001	0.431	<0.001
TC	0.416	<0.001	-0.258	0.134
HDL-C	-0.298	<0.01	-0.363	<0.05
Apo A-I	0.053	0.654	-0.217	0.211
Apo A-II	0.379	<0.001	-0.013	0.940
Apo B	0.528	<0.001	0.004	0.983

r; coefficient of correlation p; level of significance

analyzed immediately. Serum leptin concentrations were determined by radioimmunoassay (RIA) using a human leptin RIA kit (Linco Research, St. Charles, MO) (11). The assay sensitivity limit was 0.5 ng/ml in serum. The within assay coefficient of variation ranged from 3.4% to 8.3%, and the between assay coefficient of variation ranged from 3.6% to 6.2%. Results were reported as ng/ml human leptin. Serum total cholesterol (TC) and high density lipoprotein cholesterol (HDL-C) were determined enzymatically using commercial enzyme kits (Wako, Tokyo, Japan and Daiichi Kagaku, Tokyo, Japan, respectively). Serum apolipoprotein A-I (Apo A-I), apolipoprotein A-II (Apo A-II), and apolipoprotein B (Apo B) were determined by the turbidimetric immunoassay (TIA) using apolipoprotein TIA kits (Daiichi Kagaku, Tokyo, Japan) (12). The interassay coefficient of variation varied between 1.0% and 3.7% for normal levels for various apolipoproteins. The results were reported as mg/dl TC, HDL-C, Apo A-I, Apo A-II, and Apo B, respectively. Serum TC, HDL-C, Apo A-I, Apo A-II, and Apo B were measured by Hitachi 7170 Type Autoanalyzer.

Statistical analysis

Unpaired analyses of results by Wilcoxon rank sum test were performed to assess whether differences were significant. The correlation coefficient (r) and statistical significance of the correlation (p value) between serum leptin concentration and BMI, percent body fat, systolic blood pressure (SBP), diastolic

blood pressure (DBP), TC, HDL-C, Apo A-I, Apo A-II, and Apo B were evaluated using Spearman correlation. All analysis were conducted with the Statistical Analysis System (SAS Institute, Cary, NC). Each quantitative variable was reported as median value (range). Statistical significance was defined as p<0.05.

Results

Table 1 shows the descriptive characteristics of study subjects by sex. The median concentration of serum leptin in the 75 males was 3.3 ng/ml, as compared with 7.6 ng/ml in the 35 females [p<0.001].

Table 2 shows the correlation of serum leptin concentrations with selected variables related to arteriosclerosis. In males, BMI, percent body fat, DBP, and Apo B were strongly positively correlated [r<0.513, p<0.001] with leptin concentration. Serum leptin concentration in males were not significantly correlated with age or Apo A-I. In females, percentage of body fat was strongly positively correlated [r=0.553, P<0.001] with leptin concentration. Leptin in females was not significantly correlated with other variables. A negative correlation was observed between HDL-C and leptin in both males [r=-0.298, p<0.01] and females [r=-0.363, p<0.05], respectively.

Discussion

Leptin is detectable in serum. Circulating immunoreactive leptin was measured in humans using a newly developed RIA. Median age was not so different between the sexes, because all subjects were in the same class. The median concentration of leptin is approximately twofold higher in females compared with males, and the median percent body fat was also higher in females than in males. The criteria on the obesity defined by the Japan Society for the Study of Obesity are as follows; $24.0 \leq \text{BMI} < 26.4$ is "overweight," and $26.4 \leq \text{BMI}$ is "obesity" (13). In this study, neither overweight nor obesity was observed in females, whereas 14 males were included in the overweight group and 6 males were categorized in the obesity group. Therefore, median BMI in females was smaller than in males.

Serum leptin levels in two females were more than 20 ng/ml. Except for these 2 females, the median concentration of serum leptin in the 33 females was 6.8 ng/ml (range: 1.8-15.3 ng/ml), and the variance in females was also larger than in males (3.3 ng/ml, range: 1.2-15.9 ng/ml) [p<0.001]. Moreover, BMI in 2 females were in normal levels. It was, however, difficult to determine why the variance in females was larger than in males because of the small number of subjects in the present study.

The largest correlation observed was with percentage of body fat irrespective of sex [males; r=0.775, p<0.001, females; r=0.553, p<0.001], confirming previous reports in human subjects (4,5). Leptin levels in sera were also significantly correlated with BMI in both sexes (14). However, BMI was also correlated with serum leptin [r=0.631, p<0.001] in males but not in females in this study. Divergence of the relationship between leptin concentration and increasing BMI in females compared with that in males suggests that sex may influence serum leptin concentration independent of body composition (11). Adiposity measured as percentage of body fat, is unlikely to be the only factor that regulates leptin concentration in serum (6). Our data suggest that sex difference makes a highly significantly independent contribution to the factor of serum leptin. However,

the large difference between the sexes with respect to serum leptin concentration needs to be studied in detail with biochemical and epidemiological research.

Although a significant inverse association between HDL-C and leptin was seen, the magnitude of the correlation [males; $r = -0.298$, females; $r = -0.363$] was much smaller than that of percentage of body fat. In addition, leptin was not independently associated with a measure of HDL-C in obesity (6). Thus, further research about the relationship between serum leptin concentration and HDL-C is needed in normal-weight and obese humans.

In conclusion, serum leptin level had a strongly positive correlation with BMI, percentage of body fat, DBP, and Apo B in males, and inversely correlated with HDL-C. In females, leptin

was positively correlated with percentage of body fat alone, and inversely correlated with HDL-C. Of the significant factors on leptin levels, percentage of body fat, BMI, DBP, Apo B, and HDL-C were the most powerful and most significant factors in males, while percentage of body fat and HDL-C were the most important factors in females. In addition, a large difference between the sexes with respect to serum leptin concentration was recognized. However, it was difficult to determine why the variance in females was larger than in males. The biological activity of leptin has not yet been established in normal young adults. Further work is needed to provide a better understanding of the metabolic implications of the serum leptin concentration in humans.

References

- 1) 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: 425-32.
- 2) Pelleymounter MA, Cullen MJ, Baker MB, et al. Effects of the obese gene product on body weight regulation in ob/ob mice. *Science* 1995; 269: 540-3.
- 3) Caprio S, Tamborlane WV, Silver D, et al. Hyperleptinemia: an early sign of juvenile obesity. Relations to body fat depots and insulin concentrations. *Am J Physiol* 1996; 34: E626-30.
- 4) Maffei M, Halaas J, Ravussin E, et al. Leptin levels in human and rodent: Measurement of plasma leptin and ob RNA in obese and weight-reduced subjects. *Nature Med* 1995; 1: 953-6.
- 5) Considine RV, Sinha MK, Heiman ML, et al. Serum immunoreactive-leptin concentrations in normal-weight and obese humans. *N Engl J Med* 1996; 334: 292-5.
- 6) Ostlund RE, Yang JW, Klein S, Gingerich R. Relationship between plasma leptin concentrations and body fat, gender, diet, age, and metabolic covariates. *J Clin Endocrinol Metab* 1996; 81: 3909-13.
- 7) Zimmet P, Hodge A, Nicolson M, Gingerich RE. Serum leptin concentration, obesity, and insulin resistance in Western Samoans: cross sectional study. *Brit Med J* 1996; 313: 965-9.
- 8) Sato T. Bio-electrical impedance analysis method. *Jpn J Clin Med* 1995; 53: 179-82 (in Japanese).
- 9) Sakamoto Y, Sato T, Ohno M, Ikeda Y. Evaluation on the body fat by bioelectrical impedance analysis. *JASSO* 1992; 13: 279-80 (in Japanese).
- 10) Frohlich ED, Grim C, Labarthe DR, Maxwell MH, Perloff D, Weidman WH. Recommendations for human blood pressure determination by sphygmomanometers. *Circulation* 1988; 77: 502A-14A.
- 11) Ma Z, Gingerich R, Santiago JV, Klein S, Smith Ch, Laudt M. Radioimmunoassay of leptin in human plasma. *Clin Chem* 1996; 42: 942-6.
- 12) Noma A, Hat Y, Goto Y. Quantitation of serum apolipoprotein A-I, A-II, B, C-II, C-III and E in healthy Japanese by turbidimetric immunoassay: reference values, and age- and sex-related differences. *Clinica Chimica Acta* 1991; 199: 147-58.
- 13) Ikeda Y. Definition and diagnosis on obesity. *JASSO* 1992; 13: 64-6 (in Japanese).
- 14) Haffner SM, Gingerich RL, Miettinen H, Stern MP. Leptin concentrations in relation to overall adiposity and regional body fat distribution in Mexican Americans. *Int J Obese* 1996; 20: 904-8.