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A Study on Relation of Obesity to Serum Lipid, Leptin and Insulin Concentration in Elementary Schoolchildren*

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The purpose of this study was to investigate the relationship among plasma leptin, lipid profiles, insulin levels, and percentage of body fat of 72 schoolchildren from Obese Clinic Center in Seoul. The subjects divided into two groups : 39 obesity children [obesity index greater than 20%] who did not have a discernable medical cause of their obesity and 33 children with a obesity index less than 20%. The mean age, height, and obesity index of the subjects were 10.4 years, 144.0 cm, and 21.3%, respectively. The mean glucose and insulin levels of the obese children were 80.5 mg/dl and 13.3 μIU/mL and those of the non obese children were 82.0 mg/dl and 4.2 μIU/mL, respectively. Obese children had significantly ($p<0.05$) higher level of total cholesterol, triglyceride, VLDL-cholesterol, LDL-cholesterol, and insulin concentration than non-obese children. However obese children had a lower level of HDL-cholesterol than non-obese children. Plasma leptin level were also positively correlated with BMI ($p<0.0001$), body fat ($p<0.0001$), tricep skinfold thickness ($p<0.0001$), mid arm circumference ($p<0.0001$), and waist hip ratio ($p<0.0001$). Plasma leptin showed a significantly positive correlation with insulin ($p<0.0001$), total cholesterol ($p<0.0001$), LDL-cholesterol ($p<0.0001$), and triglyceride ($p<0.0001$) levels, however, it was negatively correlated with HDL-cholesterol ($p<0.0001$) levels. In conclusion, the concentration of leptin in the blood is significantly correlated with the amount of body fat, BMI, plasma insulin, and plasma lipid profiles.

Key words: Leptin, Insulin, Body fat, BMI, Schoolchildren, Obesity

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INTRODUCTION

The prevalence of obesity in children has increased in the last few decades. It has been supported by many studies that the obesity is an important predictor for the adulthood obesity among the older children.[1)] Moreover, overweight in children predicts a broad range of adverse health effect that are independent of adult weight.[2)] Therefore, it is important to identify the cause of obesity and to prevent obesity in childhood.

Obesity is a multi-factorial disorder with a strong genetic component. Studies with the twins[3)] and adoptees[4)] indicate that most familiar aggregation of obesity is attributable to genetic influence rather than to shared familiar environment. The recent identification and development of polymorphic markers within candidate genes, and associations of alleles at candidate loci has proved an informative and practical method fro analysing polygenic quantitative traits in human populations. The risk of becoming obese has a strong genetic component. One such gene is that for the obese (*Ob*) receptor gene.

Since the discovery of the *ob* gene and its gene product, leptin, in 1994,[5)] evidence from experimental studies has shown that leptin functions as a marker of peripheral body fat stores and that it is involved in the control of energy balance through the inhibition of food intake and the stimulation of energy expenditure.[6-9)] The role of leptin in the human remains unresolved. Leptin is secreted by the adipocyte. Many studies now have demonstrated that circulating levels of leptin in the human are related directly to parameter of obesity such as body mass index (BMI) or percent of body fat.[10-12)] Leptin levels have been

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consistently associated with body adiposity.[13] However, leptin levels are higher in females than in males, even after adjusting for greater females fatness, and this has been shown in both adult and children.[14,15] Even if elevated leptin resistance, it still may be a useful screening tool for children with early metabolic disorders. Other studies have examined the association between plasma leptin level and insulin sensitivity among the adults.[16] This relationship has not been examined among children, yet insulin resistance in children may be related to chronic disease later in life.[17,18]

The purpose of this study was to examine the relationship of plasma leptin, insulin, and lipid profiles with anthropometirc measurements in normal and obese schoolchildren.

SUBJECTS AND METHODS

1. Subjects

The subjects of this study were recruited from Obese Clinic Center in Seoul. They were a total of 72 children aged 7-12 y (40 boys and 32 girls) for whom we obtained blood samples for analysis. All volunteers for the study read and signed the informed consent. Participants were asked to maintain their normal dietary and physical activity through the duration of the study. The participants were divided into the 2 groups according to their obesity index: 39 obese children [obesity index greater than 20%] who did not have a discernable medical cause of their obesity and 33 children with a obesity index less than 20%. Obesity index [(real weight-standard weight)/standard weight×100] was calculated using the standard weight for Korean children, which was proposed by the Korean Pediatric Society in Korea and determined according to age, gender and stature in 1998.[19] Subject who had problem with blood collection were excluded from the study.

2. Methods

Anthropometric measurements

Height was measured to the nearest 0.1 cm, body weight was measured to the nearest 0.1 kg using digital scale system (HW-600., Mesmed System Co., LTD). Three measures of body composition were obtained: Total body fat percent (TBF, %), subcutaneous fat estimated from the tricep skinfold thickness (TSF, mm), and lean body mass percent (LBM, %). The tricep skinfold thickness were measured to 0.5 mm with skinfold caliper using standard anthropometric methods. For the percentage of body fat measurement, BIA (Bioelectrical Impedance Assay) method was taken using body composition analyzer (BA-200., Mesmed System Co., LTD). Waist circumference was measured at the smallest horizontal circumference between the 12th rib and the iliac crest. Hip circumference was measured around the buttocks at the maximum circumference. The waist-to-hip ratio (WHR) was determined from the waist circumference divided by the hip circumference. All measures were to minimize the error of measurements.

Biochemical analysis

(1) Blood sampling

Ten milliliters of fasting blood was taken from each subject for the determination of the following parameters; lipid profile, leptin, and insulin. The blood was collected using the Ethylendiaminetetra acetic acid (EDTA) treated tube and centrifuged at 3000 rpm for 20 minutes at 4 ℃ to separate plasma. The plasma samples were frozen under nitrogen and stored at -70 ℃ until to be analyzed.

(2) Analysis of plasma leptin

Plasma leptin concentrations were measured by radio immunoassay with reagents supplied by Linco Reaserch (St Charels, Mo, USA).[20] The range of the standard curve in this assay was 0.5-100 μg/L and the intraassay and interassay coefficients of variation (CVs) were less than 8%.

(3) Analysis of plasma insulin and glucose

The plasma insulin concentration was assayed by a standard radioimmunoassay method (Dia product, LA, USA) and plasma glucose concentration was measured by an enzymatic colorimetric method (Boehringer Mmannhem, Germany).

(4) Analysis of plasma lipid profile

Levels of plasma lipids (total cholesterol, HDL-cholesterol, and triglyceride) were measured by method using a colorimetry (Boehringer Mannhem, Germany). Very low density lipoprotein (VLDL) and low density lipoprotein (LDL) were calculated by the Fridewald law,[21] respectively.

VLDL-Cholesterol=Triglyceride÷5

LDL-Cholesterol=Total Cholesterol−(HDL− Cholesterol +VLDL-Cholesterol)

3. Statistical Analysis

SAS-PC software was used for all the analysis. Statistical significance of parametric data was assessed by Student's paired and unpaired t-test. Linear regression analysis was done using the least squares method and linear correlations were tested with the Pearson's correlation test. Pearson's correlation coefficient (r) were determined to

assess the relationship between obesity index and plasma leptin concentration for total subjects. Statistical significant difference were accepted at $p<0.05$. Values are presented mean±standard deviation.

RESULTS AND DISCUSSION

1. General Characteristics and Anthropometric Measurements

Total subjects were 72 children (Male:40, Female:32) with the mean age of 10.4±1.5 yr (range 7-12 yr). Table 1 shows that the obesity index, anthropometry and body composition by obesity index status. Obese children have significantly higher weight, mid arm circumference (MAC), WHR, TSF and % TBF than those of non-obese children. Obesity index was 38.5±11.3% in the obese group and 0.8±11.6% in the non-obese group ($p<0.001$). Comparing to the height in fifth-grade school boys which was significantly different between non-obese and obese group,[22] in this study, the height showed no difference between groups. Other studies[23,24] also found significant difference in weight, WH, and % TBF between non-obese and obese children.

Table 1. General characteristics, anthropometric measurements and body composition of the subjects

	Total (n=72)	Obese (n=39)	Non-obese (n=33)
Age (yr)	10.4±1.5[1)]	10.9±1.7	9.9±1.1
Weight (kg)*	47.0±11.8	54.4±10.6	38.5±5.9
Height (cm)	144.0±9.6	144.9±11.6	143.1±6.7
Obesity index (%)*	21.3±22.0	38.5±11.3	0.8±11.6
MAC (cm)*	24.0±6.7	28.8±3.5	18.2±4.8
WHR*	0.85±0.1	0.94±0.1	0.74±0.08
TSF (mm)*	22.8±7.8	29.2±5.0	16.1±3.1
TBF (%)*	25.9±13.4	36.7±7.4	13.3±5.2
LBM (%)	57.7±12.0	58.6±15.0	56.7±6.7

1) Values are mean±S.D.
Obesity index=[(real weight - standard weight) / standard weight×100];
MAC: mid arm circumference; WHR: waist -hip ratio;
TSF: tricep skinfold thickness; TBF: total body fat; LBM: lean body mass
* $p<0.05$: Significant difference between obese and non-obese by Student's t-test

2. Plasma Levels of Lipid Profiles, Leptin and Insulin

Statistically significant differences between obese and non-obese children for the plasma variables are indicated in the Table 2. The concentration of plasma total cholesterol (TC), triglyceride (TG), VLDL-cholesterol (VLDL-C) and LDL-cholesterol (LDL-C) in the obese-children were higher than these of non-obese children, however plasma HDL-cholesterol (HDL-C) concentration of the obese children was significantly lower compared with that of the non-obese children. Plasma leptin and insulin levels were significantly higher in the obese than the non-obese children (18.3±7.4 versus 3.9±3.0 ng/mL, 13.3±7.9 versus 4.26±3.0, respectively).

The hypertriglyceridemia, hypercholesterolemia in the obese-children were 22.9%, and 57.1%, respectively. Kim *et al.*[24] have examined the concentrations of serum TG, LDL-C, VLDL-C, glucose, and insulin in the obese children were much higher than those of non-obese children. Also, they found serum concentration of TG, LDL-C, and glucose were more closely associated with body mass index (BMI) than with WHR in the obese children.

Table 2. Plasma lipid profiles, insulin and leptin levels of subjects

	Total (n=72)	Obese (n=39)	Non-obese (n=33)
Triglyceride (mg/dl)*	90.3±56.7[1)]	116.2±62.0	60.0±28.8
Total Cholesterol (mg/dl)*	158.7±27.2	175.1±23.1	137.9±17.6
VLDL-Cholesterol (mg/dl)*	17.9±11.8	23.2±12.3	11.6±7.4
LDL-Cholesterol (mg/dl)*	84.9±36.9	108.1±25.1	57.8±29.3
HDL-Cholesterol (mg/dl)*	55.7±20.0	43.7±11.7	69.7±18.6
GOT (mg/dl)[2)]*	29.7±11.8	35.0±10.3	23.6±10.4
GPT (mg/dl)[3)]*	34.5±15.8	36.6±17.5	32.1±13.4
Glucose (mg/dl)	81.3±7.0	80.5±7.8	82.0±6.17
Insulin (ulU/mL)*	9.09±7.7	13.3±7.9	4.26±3.0
Leptin (ng/mL)*	11.6±9.2	18.3±7.4	3.9±3.0

1) Values are Mean±S.D.
2) Glutamic oxaloacetic transaminase
3) Glutamic pyruvic transaminase
* $p<0.05$: Significant difference between obese and non-obese by Student's t-test

3. Association between Leptin Concentration, Body Composition, Anthropometry, and Lipid Profiles

Correlation of plasma leptin level with anthropometric measurement and body composition in subjects

Plasma leptin level was positively correlated with BMI ($p<0.0001$), % TBF ($p<0.0001$), TSF ($p<0.0001$), MAC ($p<0.0001$), and WHR ($p<0.0001$) (Fig. 1). This indicated that the leptin levels were significantly higher in obese children than that in nonobese children ($p<0.0001$). As shown in Fig. 1, there were significant correlations between leptin concentration and body composition. Human obesity is a complex consequence of many factors. We initiated this study to evaluate the possibility that plasma leptin concentrations may be related to the differences found in intermediary metabolism and body composition. Although the pathophysiology of leptin in humans is not as simple as it seemed to be in rodent models of obesity, its role as an adipocyte-derived signaling molecule seems well established. Adipose tissue leptin mRNA concentrations and plasma leptin levels have been found to be closely correlated with the size of the adipose tissue depot.[25]

The relationship of serum leptin concentration with some measurement of adiposity (e.g., BMI, %TBF) has been demonstrated in several recent reports.[26,27] In a comparison of the relationship between leptin levels and various measures of adiposity (BMI, TBF, TSF, and WHR), it was found that TBF, TSF, and WHR measures were more strongly correlated with plasma levels of leptin. Most studies analyzing the relationships between leptin and pubertal development in humans have used BMI or other weight-dependent measures of adiposity. In adults, weight gain is almost due to increases in adiposity, whereas in growing children, increases in bone and muscle mass account for a significant proportion of weight gain. Therefore, especially when studying children, the particular technique used to measure body fat stores may affect the detections of other factors associated with leptin.

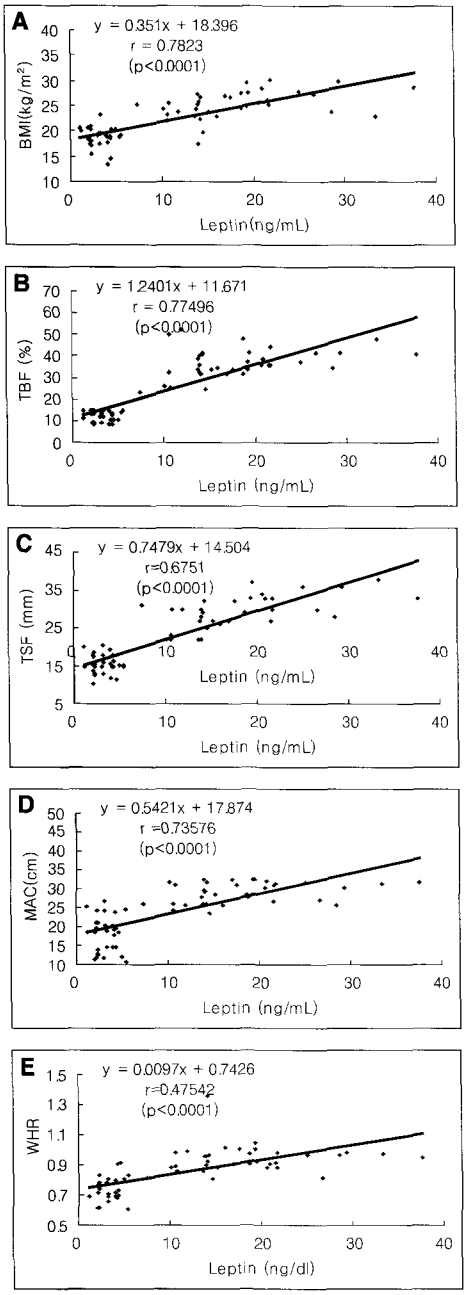

Fig. 1 Correlation of plasma leptin level and BMI (A), TBF (B), TSF (C), MAC (D), and WHR (E) in subjects

BMI: Body mass index; TBF: Total body fat; TSF: Tricep skinfold thickness; MAC: Mid arm circumference; WHR: Waist hip ratio

' *Correlation of plasma leptin with lipid profiles and insulin in subjects*

Plasma level of leptin was positively correlated with the level of insulin ($p<0.0001$), TG ($p<0.0001$), TC ($p<0.0001$) and was negatively associated with HDL-C

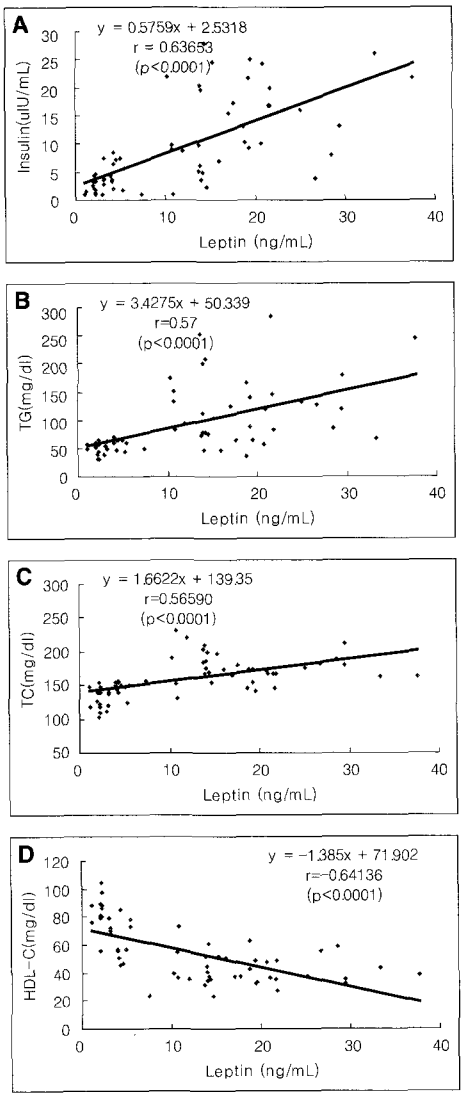

Fig. 2 Correlation of plasm insulin (A), TG (B), TC (C), HDL-C (D) concentration and leptin level in subjects

TG: Triglyceride; TC: Total cholesterol; HDL-C: High density lipoprotein cholesterol

($p<0.0001$) (Fig. 2). The relationship between plasma leptin and insulin levels is complicated and may be bi-directional. Although plasma leptin concentrations are highly correlated with obesity index, insulin levels, and insulin sensitivity in humans,[28-30] it is not yet known whether leptin has a direct effect on insulin sensitivity or if it is only a marker of obesity and related disorders. Both hormones participate in the neuro-endocrine obesity axis and the satiety control pathway, but both hormones also modulate each other's functional activities, as well as insulin resistance.[31] Berti *et al.*, provided data with evidence for a positive relation between the signaling chain of the insulin receptor and the leptin receptor. The resulting dual regulating action of leptin on glucose transport and glycogen synthesis, as well as on insulin signaling appears to be concentration-dependent mechanism. The cross-sectional survey limits for ability to evaluate the casual relationship between leptin and insulin resistance.[32] In summary, this study has demonstrated that plasma leptin levels in obese children, when expressed on the basis of BMI, is greater than age-matched non obese children as has been demonstrated by other investigators.

CONCLUSION

The purpose of this study was to investigate the relationship among plasma leptin, plasma lipid profiles, anthropometry and body composition of 72 school children recruting in obese clinic, Seoul. The subjects were recruited for studies of physiology and metabolism in 2 groups: 39 obesity children [obesity index greater than 20% group] who did not have a discernable medical cause of their obesity and 33 children with a obesity index less than 20%. Obesity index [(real weight - standard weight)/standard weight×100] was calculated using the standard weight for Korean children.

The results were as follows;

1) The mean age, body weight, height, and Obesity index were 10.4 yr, 47.0 kg, 144.0 cm, and 21.3%, respectively. Obese children were significantly higher body weight than non-obese children. Obesity index was significantly greater for obese than for non-obese children (38.5% versus 0.89%), respectively.
2) Obese children were significantly higher MAC, WHR, TSF, and TBF than non-obese children.
3) Obese children were significantly higher TC, TG, VLDL-C, LDL-C, Insulin concentration than non-obese children. However obese children had a lower HDL-C than non-obese children. Leptin was significantly greater for obese than for non-obese children (18.3 ng/mL versus 3.9 ng/mL), respectively.
4) Plasma leptin showed a significantly positive correlation with BMI ($p<0.0001$), % TBF ($p<0.0001$), TSF ($p<0.0001$), MAC ($p<0.0001$), and WHR ($p<0.0001$).
5) Therefore, these results suggest that the increase of plasma levels of leptin may related to the levels of lipid and insulin, and obesity index. plasma leptin showed a significantly positive correlation with insulin ($p<0.0001$), TC ($p<0.0001$), LDL-C ($p<0.0001$), and TG ($p<0.0001$), however, it had significantly negative correlation with HDL-C ($p<0.0001$). Interpretation of these finding would be helpful by more information about the pathophysiology of obesity and response to leptin secretion.

In conclusion, the concentration of plasma leptin is a good marker of BMI and subcutaneous body fat in both obese and non-obese children.

Literature Cited

1) Whitaker RC, Wright JA, Pepe MS, Seidel KD, Dietz WH. Prediction obesity in young adulthood from childhood and parental obesity. *New Eng J Med* 337:869-873, 1997
2) Must A, Jacque PF, Dallal GE, Bajema CJ, Dietz WH. Longterm morbidity and mortality of overweight adolescents: a follow-up of the Havard Growth Study of 1992 to 1993. *New Eng J Med* 27:1350-1355, 1992
3) Stunkard AJ, Harris JR, Pederson NL, McClearn GE. The body mass index of twin who have been reared apart. *N Eng J Med* 322:1483-1487, 1990
4) Sorensen TIA, Price RA, Stunkard AJ, Schulsinger F. Genetics of obesity in adults adoptees and their bio logical siblings. *BMJ* 298:87-90, 1989
5) Zhang Y, Proenca R, Maffei M, Barone M, leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homologue. *Nature* 372:425-432, 1994
6) Camfield LA, Smith FJ, Guisez Y, Devos R, Burn P. Signal linking adiposity and central neural network. *Science* 269:546-549, 1995
7) Halas JL, GaJIwala KS, Maffei M, Cohen S, Chait BT, Rabinowitz D, Lallone RL, Burley SK, Friedman JM. Weight-reducing effects of the plasma protein encoded by the obese gene. *Science* 269:543-546, 1995
8) Hwa JJ, Ghibaudi L, Compton D, Fawzi AB, Strader CD. Intracerebroventricualr injection of leptin increase thermogenesis and mobilizes fat metabolism in ob/ob mice. *Horm Metab Res* 29:659-663, 1996
9) Pellymounter MA, Cullen MJ, Baker MB, Hecht R, Winters

D, Boone T, Collins F. Effect of the obese gene product on body weight regulation in ob/ob mice. *Science* 269:540-543, 1995

10) Considine RV, Shinha MK, Heiman ML, Kriaucinuas A, Stephens TW, Nyce MR. Serum immunoreative-leptin concentrations in normal-weight and obese humans. *N Engl J Med* 334: 292-5, 1996
11) Rosenbaum M, Nicolson M, Hirsch J, Heymsfield SB, Gallagher D, Chu F. Effects of gender, body composition and menopause on plasma concentrations of leptin. *J Clin Endocrinol Metab* 81:3424-7, 1996
12) Ostlund RE Jr, Yang JW, Klein S, Gingerich R, Relation between plasma leptin concentration and body fat, gender, diet, age and metabolic covariates. *J Clin Endocrinol Metab* 81:1293-300, 1996
13) Thompson DB, Ravussin E, Beneett PH, Bogardus C. Structure and sequence variation at the human leptin receptor gene in lean and obese Pima Indians. *Hum Mol Genet* 6: 975-679, 1997
14) Caprio S, Tamborlane WV, Silver D, Robinson C, Leibel R, Mccarthy S, Grozman A, Belous A, Maggs D. Hyperleptinemia: an early sign of juvenile obesity. Relations to body fat depots and insulin concentrations. *Am J Physiol* 271:626-620, 1996
15) Hassink SG, Sheslow DV, Delancey E, opentanova I, Considine RV, Caro JF. Serum leptin in children with obesity: Relationship to gender and development. *Pediatrics* 98:201-203, 1996
16) Lee SK, Noh HL, Oh YJ, Kim YJ, Hong EG, Chae BN, Chung YS, Lee KW, Kim HM. Plasma Leptin, Insulin Resistance, and Obesity Index of Type 2 Diabetics and normal Subjects among Koreans. *J Kor Soc Endocrinol* 14:122-133, 1999
17) Raitakari OT, Porkka KVK, Ronnemaa T, knip M, Huari M, Akerblom HK, Viikari JSA. The role of insulin in clustering of serum lipids and blood pressure in children and adolescent. *Diabetologia* 38:1042-1050, 1995
18) Ronnemaa T, Knip M, Lautala P, Viikari J, Uhari M, Leino A, Kaprio EA, Salo MK, Dahl M, Nuutinen EM. Serum insulin and other cardiovascular risk indicators in children, adolescents and young adults. *Ann Med* 23:67-72, 1991
19) Standard weight for Korean children. The Korean Pediatric Society, 1999
20) Ma Z, Gingerich RL, Santiago JV, Klein S, Smith CH, Landt M. Radioimmunoassay of leptin in human plasma. *Clin Chem* 42:942-946, 1996
21) Friedewald WT, Levy R, Fredrickson DS. Estimation of the concentration of low density lipoprotein-cholesterol in plasma without use of the preparative ultracentrifuge. *Clin Chem* 18: 499-502, 1972
22) Lee HM, Choi KS. A study on the characteristics in lifestyle, eating habit and food preferences of overweight and obese children in Pocheon area. *J Community Nutr* 1(1):10-15, 1999
23) Kang YJ, Hong CH, Hong YJ. The percentage of childhood and adolescent obesity over the last 18 years in Seoul area. *Korean J Nutr* 30(7):832-839, 1997
24) Kim SH, Kim GE, KIm SY. A Study on relations of obesity to serum lipid and insulin concentration in elementary school children. *Korean J Nutr* 31(2):159-165, 1998
25) Considine RV, Considine EL, Williams CJ. Evidence against either a premature stop codon or the absence of obese gene mRNA in human obesity. *J Clin Invest* 95:2986-2988, 1995
26) Salbe AF, Nicolson M, Ravunssin E. Total energy expenditure and the level of physical activity correlate with plasma leptin concentration in five-year-old children. *J Clin Invest* 99: 592-595, 1997
27) Weigle DS, Bukowsk TR, Forster DC, Holderman S, Kramer JM, Lasser G. Recombinant ob protein reduces feeding and body weight in the ob/ob mouse. *J Clin Invest* 96:2065-70, 1995
28) Zhang Y, Proenca R, Maffel M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homologue. *Nature* 372:425-432, 1994
29) Pelleymounter MA, Cullen MJ, Baker MB, Hecht R, Winster D, Boone T, Collins F. Effects of the obese gene product on body weight regulation in ob/ob mice. *Science* 269: 540-543, 1995
30) Rosenbaum M, Nicolson M, Hirsch J, Heymsfield SB, Gallagher D, Chu F, Leibel. Effects of gender, body composition and menopause on plasma concentrations of leptin. *J Clin Endocrinol Metab* 81:3424-3427, 1996
31) Kolaczynski JW, Nyce MR, Considine RV, Boden G, Nolan JJ, Henry R, Mualiar SR, Olefsky J, Caro JF. Acute and chronic effect of insulin of leptin production in human. Studies *in vivo* and *in vitro*. *Diabetes* 45:699-701, 1996
32) Zimmet P, Hodge A, Nicolson M, Staten M, de Courten M, Moore J, Morawiecki A, Lubina J, Collier G, Alberti G, Dowse G. Serum leptin concentration obesity and insulin resistance in Western Samoans: cross sectional study. *Br Med J* 313: 965-969, 1996