Insulin concentration is the main predictor of leptin level: a homogenous type 2 diabetes cohort study in Taiwan

Chung-Hua Hsu[1,2]; Su-Ching Lin, Kung-Chang Hwang[3], Pesus Chou[1], Ying-Li Liao[4]
Community Medicine Research Center and Institute of Public Health, National Yang-Ming University, Taipei [1] Branch of Chinese Medicine, Taipei City Hospital,[2] Department of Pediatrics,[3] Department of Endocrinology[4] Taipei Hospital, Taiwan

Abstract
We hypothesized that there would be strong association between insulin level and leptin level, and attempted to examine factors affecting plasma leptin level among the homogeneous Type 2 diabetes subjects. The study was conducted from July 2005 through September 2005 in the Taipei Hospital, Taiwan. From 1356 registered diabetes patients, 120 subjects who met the criteria of (1) aged between 20 and 75 years, (2) being Chinese, (3) having Type 2 diabetes for more than one year, (4) having been taking gliclazide and metformin for more than 6 months, were enrolled for this study. Factors affecting plasma leptin level are determined using multiple linear regression analysis. The coefficients obtained by multiple regression analysis with stepwise method showed that gender (β=-0.49, $p<0.001$), insulin level (β=0.46, $p<0.001$) and body mass index (BMI)(β=0.21, $p=0.003$) were the main predictors of leptin concentrations for all type 2 diabetic subjects; insulin level (β=0.50, $p<0.001$), BMI (β=0.42, $p<0.001$) and triglyceride (β=0.17, $p=0.03$) were the main predictors for type 2 diabetic men; and insulin level (β=0.68, $p<0.001$) was the only predictor for type 2 diabetic women. These initial findings seem to indicate that insulin concentration is the main predictor of leptin level in both male and female type 2 diabetes subjects.

Keyword: *Insulin, Leptin, Type 2 diabetes*

Introduction

Leptin is a hormone produced in adipocytes.[1] It has been suggested that leptin directs metabolic fuels towards utilization and away from storages.[2] The development of Type 2 diabetes in association with obesity, hyperinsulinemia and insulin resistance have been demonstrated. Obesity is associated with a marked increase in circulating leptin concentration.[3-4] However, plasma leptin displays a strong correlation with insulin concentration, insulin resistance, metabolic syndrome, dyslipidemia, even after controlling for measures of body fat mass.[5-8]

The different severity or condition of type 2 diabetes might result in different degree of insulin concentration and resistance. Many studies have demonstrated that taking oral antidiabetic drugs might change the plasma leptin concentration in type 2 diabetes.[9-10] To avoid confounders and bias due to different severity of diabetes, we examined a homogenous Chinese cohort who had had type 2 diabetes for more than one year, had been taking gliclazide and metformin for more than six months, and were 20-75 years old.

We hypothesized that there would be strong association between insulin level and leptin level, and attempted to examine factors affecting plasma leptin level among the homogeneous Type 2 diabetes subjects.

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Correspondence to: Dr. Ying-Li Liao, Department of Endocrinology, Taipei Hospital, Taiwan. Tel: 886-2-22765566 ext 1131. Fax: 886-2-22765590, e-mail: chmed44@ptph.doh.gov.tw

Research design and method

Study population

The trial was conducted from July 2005 through September 2005 in the Taipei Hospital, Taiwan. A total of 1356 registered diabetic patients were screened, with 186 meeting the inclusion criteria /exclusion criteria (Table 1). A letter explaining the purpose of the study was sent to the 186 patients inviting their participation. Among them, 142 accepted the invitation. We excluded 22 subjects after approaching them, and in the end, 120 subjects (63 males and 57 females) were enrolled and signed an informed consent. The protocol was approved by the Human Ethics Committee of Taipei Hospital.

Assessment

Multiple linear regression analysis was employed to

Table 1: Inclusion and exclusion criteria.

Inclusion criteria.
(1) Age between 20 and 75 years old
(2) Chinese populatio
(3) Type 2 diabetes more than one year
(4) Taking gliclazide and metformin more than six months
Exclusion criteria
(1) GOT, GPT＞80 U/L, serum creatinine ＞2.0 mg/dl
(2) Prolaction or pregnancy women
(3) Heart failure, AMI stroke and heavy injury diseases
(4) Any other conditions not suitable for trial as evaluated by the physician.

Table 2: Multiple regression analysis of serum leptin with stepwise method.

	All		Male		Female	
Factors	β	p	β	p	β	p
Gender (male=1/female=0)	-0.49*	<0.001				
Basic data						
Age	0.06	0.43	0.006	0.94	-0.001	0.99
Body mass index	0.21*	0.003	0.50*	<0.001	0.10	0.35
Waist circumflex	0.08	0.54	0.17	0.28	0.07	0.57
Systolic blood pressure	0.05	0.47	0.02	0.83	0.05	0.63
Diastolic blood pressure	0.06	0.39	0.06	0.45	0.08	0.47
Fasting serum factors						
glucose	0.03	0.64	-0.03	0.68	0.01	0.90
HbA1c	-.004	0.95	-0.01	0.87	-0.01	0.90
Insulin	0.46*	<0.001	0.42*	<0.001	0.68*	<0.001
Plasma lipoprotein						
Fasting triglyceride	-0.03	0.63	0.17*	0.03*	-0.03	0.78
Fasting cholesterol	0.04	0.53	0.10	0.27	0.05	0.64
HDL-cholesterol	0.07	0.29	0.11	0.10	0.09	0.38
LDL-cholesterol	0.02	0.70	0.03	0.69	0.06	0.57

* predictor of model.

Table 3: Multiple regression analysis of serum leptin with stepwise method.

	All		Male		Female	
Factors	β	p	β	p	β	p
Gender (male=1/female=0)	-0.49*	<0.001				
Basic data						
Age	0.06	0.43	0.006	0.94	-0.001	0.99
Body mass index	0.21*	0.003	0.50*	<0.001	0.10	0.35
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Diastolic blood pressure	0.06	0.39	0.06	0.45	0.08	0.47
Fasting serum factors						
glucose	0.03	0.64	-0.03	0.68	0.01	0.90
HbA1c	-.004	0.95	-0.01	0.87	-0.01	0.90
Insulin	0.46*	<0.001	0.42*	<0.001	0.68*	<0.001
Plasma lipoprotein						
Fasting triglyceride	-0.03	0.63	0.17*	0.03*	-0.03	0.78
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* predictor of model.

determine the factors affecting plasma leptin level. Measurements including blood pressure, BMI, fasting glucose, hemoglobulin A1c% (HbA1c), insulin, and plasma lipoproteins (triglyceride, cholesterol, cholesterol-HDL (HDL) and cholesterol-LDL (LDL)) were also analyzed. All measurements were made at 0800-0900 after an overnight fast using standardized methods. Height was measured with a wall-mounted stadiometer to the nearest 0.1 cm, weight was measured on a calibrated balance beam scale to the nearest 0.1 kg, and BMI was calculated (BMI= body weight (BW) / height (kg / m2)). A mercury sphygmomanometer with standard cuff was employed to measure the indirect auscultatory arterial blood pressure taken from the right arm with subjects in seated position.

Analysis of blood samples

A sample of whole blood was drawn and centrifuged at 4°C, and a 1-ml aliquot of serum was rapidly frozen (-80°C) for subsequent hormone analysis. The plasma leptin concentration was measured with a human leptin RIA assay (Linco Research, Inc, St. Charles, MO, USA). The limit of sensitivity is 0.5 ng/ml. There is no cross-reactivity with human insulin and pro-insulin. Plasma insulin levels were measured using a commercially available RIA (Linco Research, Inc). Fasting glucose, HbA1c, cholesterol, triglyceride, LDL and HDL were analyzed at the clinical laboratories at the hospital.

Statistical analysis

The Student t-test was employed to analyze the comparisons between male and female. Multiple linear regression analysis with stepwise method was performed on leptin concentrations. All p values were two-tailed and α level of significance was set at 0.05. The data were analyzed with

SPSS software (version11.5).

Results

Demographics

Table 2 shows the demographic and biochemical characterics of the study population according to gender. As can be seen, there is significant gender difference in leptin level ($p<0.001$), but not in BMI ($p=0.74$) or insulin level ($p=0.63$).

Coefficients of linear multiple regression on leptin level

Table 3 shows the coefficients obtained by linear multiple regression on leptin level with stepwise method. As can be seen, gender ($\beta=-0.49$, $p<0.001$), insulin level ($\beta=0.46$, $p<0.001$) and BMI ($\beta=0.21$, $p=0.003$) were the main predictors of leptin concentrations in all type 2 diabetic subjects; insulin level ($\beta=0.42$, $p<0.001$), BMI ($\beta=0.50$, $p<0.001$) and triglyceride ($\beta=0.17$, $p=0.03$) were the main predictors in type 2 diabetic male; and insulin level ($\beta=0.68$, $p<0.001$) was the only predictor in type 2 diabetic female.

Conclusion

Previous research has explored the regulators of leptin level in type 2 diabetic subjects[12-14] Many studies have found that leptin levels were influenced by different factors such as adipose fat mass, waist circumflex, BMI, insulin or insulin resistance.[1,-5-7, 12-14] The strong association between insulin and leptin has also been demonstrated.[15-19] Moreover, it has been reported that taking oral antidiabetic drugs might change the plasma leptin concentration in type 2 diabetes patients.[9-10] Hence, this study attempted to examine the factors affecting the plasma leptin level in diabetic patients taking the same oral antidiabetic drugs. To avoid possible bias or confounder, we chose a homogenous cohort made up of Chinese Type 2 diabetes patients who had been taking the same oral hypoglycemic agents for more than 6 months. Patients with abnormal renal function, liver function impairment, heart failure, stroke and heavy injuries as well as prolactating or pregnant women were excluded.

Our findings reconfirmed that insulin level, gender and BMI are the main predictors of leptin level in this homogenous diabetes cohort. For female diabetic subjects, insulin level is the only predictor of leptin level. This highlights the role of insulin concentration as the main regulator of leptin level.

As mentioned above, our results reveal gender difference in leptin concentrations. It was found that females (8.3(3.9) ng /ml) have higher leptin concentration than males (4.4(2.4) ng) ($p<0.001$). This is consistent with the findings of many previous studies on leptin level among normal population.[20-25] Menenodez et al. reported that leptin secretion from omental adipose tissue in vitro was higher in samples from women than those from men, and that the secretion is not correlated with BMI.[24] They attributed the higher circulation leptin levels in female to an intrinsic property of female adipocytes, which secretes more leptin than male tissue. The study by Garcia et al. of 789 children showed that girls have higher leptin level than boys, but obviously not due to adiposity.[26] The same results were also found among newborns.[27, 28] The sex differences in fetal leptin levels are less likely to be due either to body fat content or different hormone status, but it may reflect genetic differences between male and female. The biological reasons behind these differences merit further study.

Results of this study showed that BMI has less effect on leptin level in females, indicating different regulators of leptin concentration at work in diabetic subjects of different sex. It may be due to the body build of females of the cohort. The smaller WC ($p=0.002$) among the female diabetic subjects implies less adipose tissues for leptin secretion. Thus, insulin concentration plays a more important role than BMI in regulating leptin levels among females.

Although fasting leptin levels are found to be linearly related to body fat mass, there is much evidence supporting the statement that changes in insulin secretion and glucose metabolism are the major mediators of leptin production by adipose tissue.[29-30] *In-vitro* studies have demonstrated that insulin can increase metabolism of glucose, which in turn mediates leptin gene expression and leptin secretion. Insulin level can also stimulate the transcriptional activity of the leptin promoter.[32] This study reconfirmed the above findings that insulin is the main predictor of leptin levels in type 2 diabetes subjects.

In conclusion, this study demonstrated that insulin concentration is the main predictor of leptin level in both male and female diabetes subjects. Females have a higher leptin level than males. The biological reasons behind the results are worth further exploration.

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