Relationship Between Fasting Leptin and Insulin Concentrations in Type 2 Diabetes Mellitus Patients

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Objectives. Many researchers have found that plasma leptin concentration is associated with insulin levels in non-diabetic subjects. However, the relationship between leptin concentrations and insulin levels in type 2 diabetes mellitus (DM) patients has rarely been investigated. The aim of this study was to investigate that relationship.

Methods. Fasting plasma glucose, fasting serum insulin (FSI), leptin concentration and body mass index (BMI) were measured in 100 type 2 DM subjects (50 males and 50 females).

Results. There were no significant differences in age, BMI, fasting plasma glucose, FSI and HbA1c between male and female subjects. The mean leptin concentration of the female subjects was significantly higher than that of the male subjects (7.75 $\pm$ 6.41 *vs* 3.72 $\pm$ 3.70 ng/mL, $p < 0.01$). Using Pearson correlation analysis, leptin concentration positively correlated with BMI ($r = 0.574$, $p < 0.001$) and FSI levels ($r = 0.498$, $p < 0.001$) in the male subjects. It also positively correlated with BMI ($r = 0.605$, $p < 0.001$) and FSI levels ($r = 0.543$, $p < 0.001$) but inversely correlated with age ($r = -0.399$, $p = 0.004$) in the female subjects. After adjusting for age, BMI, and insulin modifying medications, FSI was significantly and positively related to leptin concentration ($\beta = 0.180$, $p = 0.031$) in the male subjects but not in the female subjects ($\beta = 0.131$, $p = 0.089$). BMI also positively correlated with leptin concentrations in both genders after considering the effects of FSI and other covariates.

Conclusions. Leptin concentration significantly correlated with FSI levels in men with type 2 diabetes but not in women with type 2 diabetes. The difference between the two groups may be due to age, gender effect, or other potential confounders. Further investigation is necessary. **(Mid Taiwan J Med 2003;8:1-7)**

Key words

insulin, leptin, type 2 DM

INTRODUCTION

Insulin is an important regulator of glucose homeostasis. It also plays a role in plurimetabolic syndrome, more commonly referred to as Syndrome X [1]. Type 2 diabetes mellitus (DM) is determined by genetic, and environmental factors, and insulin resistance [2,3]. In addition, insulin resistance is associated with other diseases, including obesity, hypertension, dyslipidemia, and cardiovascular disease [4,5]. Modest weight loss can reduce insulinemia, thereby contributing to a decrease in morbidity and presumably, mortality [6,7].

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Table 1. Clinical and metabolic characteristics of subjects

	Male (n = 50)	Female (n = 50)	Total (N = 100)
Age (yr)	50.48 ± 7.29	51.64 ± 7.51	51.06 ± 7.39
BMI (kg/m^2)	24.40 ± 4.05	26.59 ± 5.46	26.00 ± 4.82
FPG (mg/dL)	148.20 ± 32.04	147.76 ± 28.90	147.98 ± 30.36
FSI (μU/mL)	9.01 ± 5.72	10.68 ± 13.40	9.84 ± 10.28
HbA1c (%)	6.94 ± 1.15	6.84 ± 1.16	6.89 ± 1.15
Leptin (ng/mL)*	3.72 ± 3.70	7.75 ± 6.41	5.73 ± 5.59
Medications for DM			
Diet alone	3 (6%)	4 (8%)	7 (7%)
Sulfonylurea (S)	8 (16%)	9 (18%)	17 (17%)
Metformin (M)	2 (4%)	4 (8%)	10 (10%)
S + M	36 (72%)	32 (64%)	68 (68%)
S + M + Acarbose	1 (2%)	1 (2%)	2 (2%)
Medications for HTN			
ACEI	5 (10%)	6 (12%)	11 (11%)
AIIRB	1 (2%)	4 (8%)	5 (5%)
Diuretics	1 (2%)	0	1 (1%)
CCB	2 (4%)	4 (8%)	6 (6%)
α-blocker	2 (4%)	0	2 (2%)
β-blocker	1 (2%)	1 (2%)	2 (2%)
ACEI + α-blocker	5 (10%)	2 (4%)	7 (7%)
ACEI + β-blocker	0	1 (2%)	1 (1%)
ACEI + CCB	1 (2%)	3 (6%)	4 (4%)
CCB + α-blocker	0	2 (4%)	2 (2%)
α-blocker + AIIRB	0	1 (2%)	1 (1%)
ACEI + CCB + α-blocker	1 (2%)	0	1 (1%)
ACEI + CCB + β-blocker	1 (2%)	0	1 (1%)
Diuretic + ACEI + CCB	1 (2%)	0	1 (1%)

Values are arithmetic means ± standard deviation; DM = diabetes mellitus; BMI = body mass index; FPG = fasting plasma glucose; FSI = fasting serum insulin; HTN = hypertension; ACEI = angiotensin-converting enzyme inhibitor; AIIRB = angiotensin II receptor blocker; CCB = Ca-channel blocker. *$p < 0.01$, male *vs* female.

Leptin, the product of the *ob* gene, fluctuates considerably with body weight changes and more particularly with adiposity [8,9]. The physiological factors that modulate plasma leptin levels include sex, body fat, exercise, and fluctuations in caloric supply. Hormones that effect leptin production include insulin, glucocorticoids, sex steroids, growth hormone, somatostatin, and insulin-like growth factor I [10-12]. End-stage renal disease which is a catabolic and anorectic state, is associated with marked elevation in leptin levels [13,14]. A current hypothesis holds that leptin is a "lipostate", regulating appetite through a negative feedback mechanism, and may counteract the anabolic fat-storing effects of insulin. Leptin has been shown to be positively associated with insulin resistance [15] and insulin concentrations [16] among non-diabetic subjects. However, the relationship between leptin and insulin concentrations in type 2 DM has rarely been investigated. The purpose of this study was to investigate the relationship.

MATERIALS AND METHODS

Subjects

We recruited 100 type 2 DM subjects (50 males and 50 females) from the diabetic clinic at the China Medical College Hospital. Type 2 DM was diagnosed according to the 1985 World Health Organization criteria [17]. Subjects who were being treated with glucocorticoids, sex steroids, growth hormone, somatostatin, or insulin, had a history of thyroid disease or serum creatinin levels >1.5 mg/dL were excluded [10-14]. All subjects were being treated with diet alone or diet plus oral hypoglycemic agents when

Table 2. Pearson's correlation coefficient (*r*) of leptin with selected variables

	Male (n = 50)	Female (n = 50)	Total (N = 100)
Age	0.060 ($p = 0.678$)	−0.399 ($p = 0.004$)	−0.182 ($p = 0.070$)
BMI	0.574 ($p < 0.001$)	0.605 ($p < 0.001$)	0.593 ($p < 0.001$)
FPG	0.174 ($p = 0.227$)	−0.177 ($p = 0.218$)	−0.038 ($p = 0.706$)
FSI	0.498 ($p < 0.001$)	0.543 ($p < 0.001$)	0.522 ($p < 0.001$)
HbA_{1c}	0.199 ($p = 0.166$)	−0.068 ($p = 0.637$)	0.011 ($p = 0.915$)

BMI = body mass index; FPG = fasting plasma glucose; FSI = fasting serum insulin.

the study began. Thirty-eight percent of the type 2 diabetic subjects also had hypertension and were being treated with anti-hypertensive agents (Table 1).

Measurements

The weight and height of each DM control subjects who had fasted overnight were recorded at our diabetic clinic. Blood samples were obtained between 8:00 and 9:00 A.M. after an overnight fast. BMI was calculated as weight (kilogram) divided by height ($meter^2$). The glycated hemoglobin A1c (HbA_{1c}) levels 3 months preceding the study were recorded. Plasma glucose concentration was assayed by the glucose oxidase method (Astra-8, Beckman, Calif, USA). HbA1c levels were measured using ion-exchange HPLC (HLC-723 GHbV, Tosoh, Tokyo, Japan). Serum was stored at −70°C until assay of the leptin and insulin concentrations. The serum insulin concentration was determined using a commercial radioimmunoassay (RIA) kit (Diagnostic Products Corp., Los Angels, Calif, USA). The inter-assay coefficient of variation (CV) of insulin was 8.7% and the intra-assay CV was 3.5%. Serum leptin concentration was measured using a commercial RIA kit Linco Research, St. Louis, Mo, USA). The inter-assay CV of leptin was 6.5% and the intra-assay CV was 3.6%.

Statistical Analysis

All data are presented as mean ± standard deviation (SD). The differences in the clinical and metabolic variables between male and female subjects were compared by Student's *t* test. The correlation between leptin and other variables was assessed by Pearson correlation coefficients. Independent associations of leptin concentration with other variables were analyzed by multiple linear regression. A *p* value of less than 0.05 was considered statistically significant. All statistical analyses were carried out by SPSS IX software.

RESULTS

The clinical and metabolic characteristics of the subjects and medications for DM and hypertension control are shown in Table 1. There were no significant differences in age, BMI, fasting plasma glucose, FSI and HbA_{1c} between male and female subjects. The mean leptin concentration of the female subjects was significantly higher than that of the male subjects (7.75 ± 6.41 *vs* 3.72 ± 3.70 ng/mL, $p < 0.01$).

The association between leptin concentration and selected variables is presented in Table 2. Leptin concentration positively correlated with BMI ($r = 0.574$, $p < 0.001$) and FSI levels ($r = 0.498$, $p < 0.001$) in the male subjects. It also positively correlated with BMI ($r = 0.605$, $p < 0.001$) and FSI levels ($r = 0.543$, $p < 0.001$) but inversely correlated with age ($r = -0.399$, $p = 0.004$) in the female subjects. In all of the subjects, leptin concentration positively correlated with BMI ($r = 0.593$, $p < 0.001$) and FSI levels ($r = 0.522$, $p < 0.001$).

We evaluated the association between leptin concentrations and FSI levels of the covariates using multiple linear regression analyses in both genders (Table 3). After adjusting for age, BMI, and insulin modifying medications, including metformin, sulfonylurea, acarbose, ACEI, AIIRB, diuretics, β-blocker and α-blocker, FSI was found to be significantly and positively related to leptin concentration ($\beta = 0.180$, $p = 0.031$) in the male subjects but not in

Table 3. Multiple linear regression analysis of selected variables with leptin values

	Male (n = 50)			Female (n = 50)		
	β	SE	p	β	SE	p
FSI	0.204	0.080	0.014	0.146	0.063	0.024
BMI	0.406	0.112	0.001	0.517	0.153	0.002
	F = 16.44 total R^2 = 41.2%			F = 17.89 total R^2 = 43.2%		
Adjusted for BMI and age						
FSI	0.193	0.079	0.019	0.133	0.074	0.081
BMI	0.438	0.144	<0.001	0.512	0.156	0.002
Age	0.075	0.058	0.204	−0.041	0.122	0.738
	F = 11.67 total R^2 = 43.2%			F = 11.74 total R^2 = 43.4%		
Adjusted for BMI, age and insulin modifying medications						
FSI	0.180	0.081	0.031	0.131	0.075	0.089
BMI	0.477	0.122	<0.001	0.508	0.158	0.002
Age	0.106	0.067	0.121	−0.048	0.125	0.703
Medication*	−2.296	2.465	0.357	1.021	3.042	0.739
	F = 8.95 total R^2 = 44.3%			F = 8.66 total R^2 = 43.5%		

SE = standard error; BMI = body mass index; FSI = fasting serum insulin. *Medications includes metformin, sulfonylurea, acarbose, ACEI, AIIRB, diuretics, β-blocker and α-blocker.

the female subjects (β= 0.131, p = 0.089). BMI also positively correlated with leptin concentration in male (β= 0.477, p < 0.001) and female (β= 0.508, p = 0.002) subjects after considering the effects of FSI and other covariates.

DISCUSSION

In this study, we found that leptin concentrations of the female subjects were significantly higher than those of the male subjects, results which are consistent with previous studies [9,16]. The mechanism of this gender difference has not been completely elucidated. The different effects of androgens and estrogens on leptin production of adipocytes [11,18] and the difference in fat distribution between men and women [19,20] may be contributed to gender differences.

Leptin concentration positively correlates with the percentage of body fat and BMI [8,9]. This relationship was confirmed by our study. It has been shown that sustained hyperinsulinemia stimulates leptin messenger RNA expression [21,22] although acute changes in insulin levels do not seem to affect the expression of leptin [23]. However, the study by Couillard et al demonstrated a positive association between insulin and leptin [24]. In fact, increases in insulin have been shown to increase leptin levels in obese males in physiological amounts [25,26]. In type 2 DM, insulin resistance alone will not result in hyperglycemia without at least some degree of impaired β-cell function. This concept is supported by data from the United Kingdom Prospective Diabetic Study (UKPDS), which supported that β-cells in patients with type 2 diabetes are already reduced by approximately 50% at the time of diagnosis [27].

Age inversely correlated with leptin concentration in our female subjects with type 2 diabetes but not in the male subjects according to Pearson analysis. However, after multiple linear regression analysis, no correlation between age and leptin concentration in female subjects was found. The reported decline in plasma leptin concentrations probably reflects the combined effects of age and estrogen deficiency [11,28]. The relationship between age and leptin concentration in both genders in our study differed from previous reports. Sample size, age distribution of selected samples, medications and other potential confounders may have contributed to the differences. In addition, after adjusting for age and BMI, the relationship between FSI levels and leptin concentration in our female subjects was not statistically significant. Age and gender effects, such as leptin decline in menopausal

women due to estrogen deficiency, may play a role in this change.

In our type 2 DM subjects, some of the insulin modifying medications given for DM and hypertension control may have affected insulin concentration or sensitivity. We cannot exclude the effects of the medications on leptin concentration, although there were no significant differences between the non-medication group (n = 5) and medication group (n = 95) in leptin concentration. Therefore, in multiple linear regression analyses we adjusted for insulin modifying medications, including metformin, sulfonylurea, acarbose, ACEI, AIIRB, diuretics, β-blocker and α-blocker. The relationship between leptin concentration and FSI levels did not change in either gender.

In conclusion, we found that plasma leptin concentration is significantly related to insulin concentration in the male subjects with type 2 diabetes but not in the female diabetics. Leptin may be involved in regulating insulin resistance and may play an important role in the pathogenesis of type 2 DM. Further investigation to elucidate the relationship among leptin concentration, age, gender effect, and insulin resistance is necessary.

REFERENCES

1. Reaven GM. Role of insulin resistance in human disease. *Diabetes* 1988;37:1495-507.
2. DeFronzo RA. Lilly lecture 1987. The triumvirate: beta-cell, muscle, liver. A collusion responsible for NIDDM. [Review] *Diabetes* 1988;37:667-87.
3. DeFronzo RA, Bonadonna RC, Ferrannini E. Pathogenesis of NIDDM. A balance overview. *Diabetes Care* 1992;15:318-68.
4. Serrano Rios M. Relationship between obesity and the increased risk of major complications in non-insulin-dependent diabetes mellitus. [Review] *Eur J Clin Invest* 1998;28(Suppl 2):14-8.
5. Stern MP. Do non-insulin-dependent diabetes mellitus and cardiovascular disease share common antecedents? [Review] *Ann Intern Med* 1996;124:110-6.
6. Goldstein DJ. Beneficial health effects of modest weight loss. [Review] *Int J Obes Metab Disord* 1992;16:397-415.
7. Wing RR, Jeffery RW. Effect of modest weight loss on changes in cardiovascular risk factors: are there differences between men and women or between weight loss and maintenance? *Int J Obes Relat Metab Disovd* 1995;19:67-73.
8. 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. *Nat Med* 1995;1:1155-61.
9. 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.
10. Masuzaki H, Ogawa Y, Hosoda K, et al. Glucocorticoid regulation of leptin synthesis and secretion in humans: elevated plasma leptin levels in Cushing's syndrome. *J Clin Endocrinol Metab* 1997; 82:2542-7.
11. Elbers JM, Asscheman H, Seidell JC, et al. Reversal of the sex difference in serum leptin levels upon cross-sex hormone administration in transsexuals. *J Clin Endocrinol Metab* 1997;82:3267-70.
12. Donahoo WT, Jensen DR, Yost TJ, et al. Isoproterenol and somatostatin decrease plasma leptin in humans: a novel mechanism regulating leptin secretion. *J Clin Endocrinol Metab* 1997;82:4139-43.
13. Sharma K, Considine RV, Michael B, et al. Plasma leptin is partly cleared by the kidney and is elevated in hemodialysis patients. *Kidney Int* 1997;51:1980-5.
14. Grinspoon S, Gulick T, Askari H, et al. Serum leptin levels in women with anorexia nervoxa. *J Clin Endocrinol Metab* 1996;81:3861-3.
15. Haffner SM, Miettnen H, Mykkanen L, et al. Leptin concentrations and insulin sensitivity in normoglycemic men. *Int J Obes* 1997;21:393-9.
16. Dagogo-Jack S, Fanelli C, Paramore D, et al. Plasma leptin and insulin relationships in obese and nonobese humans. *Diabetes* 1996;45:695-8.
17. WHO Study Group. Diabetes mellitus. Technical report series no. 727. Geneva: World Health Organization, 1985.
18. Jockenhovel F, Blum WF, Vogel E, et al. Testosterone substitution normalizes elevated serum leptin levels in hypogonadal men. *J Clin Endocrinol Metab* 1997;82:2510-3.
19. Hu FB, Chen C, Wang B, et al. Leptin concentration in relation to overall adiposity, fat distribution, and blood pressure in a rural Chinese population. *Int J Obes Relat Metab Disord* 2001;15:121-5.
20. Bennett FI, McFarlane-Anderson N, Wilks R, et al. Leptin concentration in women is influenced by

regional distribution of adipose tissue. *Am J Clin Nutr* 1997;66:1340-4.

21. Kolaczynski JW, Considine RV, Ohannesian J, et al. Responses of leptin to shot-term fasting and refeeding in humans: a link with ketogenesis but not ketones themselves. *Diabetes* 1996;45:1511-5.
22. Kolaczynski JW, Ohannesian J, Considine RV, et al. Responses of leptin to shot-term and prolonged overfeeding in humans. *J Clin Endocrinol Metab* 1996;81:4162-5.
23. Vidal H, Auboeuf D, De Vos P, et al. The expression of ob gene is not acutely regulated by insulin and fasting in human abdominal subcutaneous adipose tissue. *J Clin Invest* 1996;98:251-5.
24. Couillard C, Mauriege P, Prud'homme D, et al. Plasma leptin concentrations: gender differences and association with metabolic risk factors for cardiovascular disease. *Diabetologia* 1997;40:1178-84.
25. Boden G, Chen X, Kolaczynski JW, et al. Effects of prolonged hyperinsulinemia on serum leptin in normal human subjects. *J Clin Invest* 1997;100:1107-13.
26. Saad MF, Khan A, Sharma A, et al. Physiological insulinemia acutely modulates plasma leptin. *Diabetes* 1998:47:544-9.
27. UK Prospective Diabetes Study (UKPDS) Group: Intensive blood-glucose control and sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). *Lancet* 1998;352:837-53.
28. Ostlund RE Jr, Yang JW, Klein S, et al. Relation between plasma leptin concentration and body fat, gender, diet, age, and metabolic covariates. *J Clin Endocrinol Metab* 1996;81:3909-13.

第2型糖尿病病人瘦素與胰島素濃度的關係

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目的 許多研究指出瘦素和胰島素濃度在非糖尿病病人呈正相關，但在第2型糖尿病病人，此種相關性較少人報告。本研究旨在探討第2型糖尿病病人，其瘦素和空腹血清胰島素的關係。

方法 一百名糖尿病病人(男女各半)納入本研究。測量其身體質量指數、空腹血糖、血清胰島素及瘦素，以統計方法觀察其相關性。

結果 在第2型糖尿病病人中，女性病人的瘦素平均值較男性爲高(7.75 ± 6.41 *vs* 3.72 ± 3.70 ng/mL，$p < 0.01$)。男性病人的瘦素濃度和身體質量指數($r = 0.574$，$p < 0.001$)及空腹血清胰島素($r = 0.498$，$p < 0.001$)呈顯著正相關；而女性病人的瘦素濃度和身體質量指數($r = 0.605$，$p < 0.001$)及空腹血清胰島素($r = 0.543$，$p < 0.001$)亦呈顯著正相關，但和年齡($r = -0.399$，$p = 0.004$)呈顯著負相關。在線性複迴歸分析中，經校正年齡、身體質量指數及會改變胰島素濃度或抗性的藥物後，瘦素與空腹血清胰島素在男性第2型糖尿病病人呈顯著獨立正相關，但在女性第2型糖尿病病人卻不呈顯著相關。

結論 瘦素與空腹血清胰島素在男性第2型糖尿病病人呈顯著獨立正相關，但在女性第2型糖尿病病人卻不呈顯著相關。年齡、性別差異及其它潛在因素，均可能影響到瘦素與空腹血清胰島素的關係。胰島素抗性、瘦素、年齡及性別差異在第2型糖尿病的關係，仍需進一步探討。（中台灣醫誌 **2003;8:1-7**）

關鍵詞

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