Renal function and insulin resistance as determinants of plasma leptin levels in patients with NIDDM

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Summary Plasma leptin level is known to correlate with the degree of obesity. To determine the influences of renal function and insulin resistance on plasma leptin concentrations, we measured plasma leptin concentrations and performed the euglycaemic hyperinsulinaemic clamp studies in 57 patients with non-insulin-dependent diabetes mellitus with a wide range of renal function. In simple regression analyses, plasma leptin concentration showed significant positive correlations with percentage of body fat measured by dual energy X-ray absorptiometry, body mass index, waist to hip ratio and fasting plasma insulin. Leptin level was higher in females than males.

Multiple regression analyses indicated that percent body fat, waist to hip ratio, plasma insulin, gender and renal function (1/creatinine), but not insulin sensitivity, were significant and independent determinants of plasma leptin level. These results suggest that plasma leptin level is regulated or affected by multiple factors including renal function. Insulin resistance appeared to increase leptin levels indirectly by raising plasma insulin. [Diabetologia (1997) 40: 676–679]

Keywords Leptin, insulin, body fat, non-insulin-dependent diabetes mellitus, renal function.

Leptin is a newly found hormone secreted by adipocytes [1]. It is the *ob* gene product which regulates food intake and thermogenesis [2–4]. The *ob/ob* mouse has a mutation in the *ob* gene [1], and *db/db* mice have a mutation in the gene coding for the leptin receptor [5, 6]. Therefore, leptin could play an important role in the pathogenesis of obesity and diabetes mellitus. In humans, however, the majority of obese subjects have elevated plasma leptin concentrations [7–9], suggesting the presence of leptin resistance [10]. The mechanisms regulating plasma leptin level or leptin resistance are not fully understood. Body fatness was one of the important determinants of

plasma leptin levels [7–9]. Some studies showed that insulin infusion increased plasma leptin concentration [11, 12], although other studies [13] did not. One report [13] indicated that insulin resistance was associated with elevated plasma leptin concentrations independent of body fat, whereas more recent studies [14, 15] failed to detect the independent contribution of insulin sensitivity or body fat distribution, an indicator of insulin resistance [16], to plasma leptin concentration. In addition, there is a possibility that renal function may play an important role in the regulation of plasma leptin concentration. Renal impairment results in insulin resistance and hyperinsulinaemia [17]. Also, the kidney is among the organs expressing leptin receptors [18], although their functions are unknown at present. To answer these controversies and to evaluate the possible impact of renal function on leptin, we performed the euglycaemic hyperinsulinaemic clamp in 57 patients with non-insulin-dependent diabetes mellitus (NIDDM). The results suggest that: 1) both total mass and distribution of body fat are important determinants of leptin levels; 2) leptin

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Abbreviations: NIDDM, Non-insulin-dependent diabetes mellitus; WHR, waist to hip ratio.

Table 1. Clinical characteristics of the subjects and their correlation with plasma leptin levels

Variables	Mean $\pm$ SEM	Range	Correlation with leptin	
			<i>r</i> -value	<i>P</i> -value
Plasma leptin (ng/ml)	6.05 $\pm$ 0.77	1.3–30.9	–	–
Age (years)	56.8 $\pm$ 1.3	21–71	0.134	0.3190
Duration of diabetes (years)	12.4 $\pm$ 1.3	0.1–51	0.109	0.4175
Smoking index (cigarette-years)	544 $\pm$ 83	0–2600	0.114	0.4004
Systolic blood pressure (mm Hg)	141 $\pm$ 4	92–242	0.156	0.2470
Diastolic blood pressure (mm Hg)	76 $\pm$ 2	56–112	–0.047	0.7257
Percent body fat	23.1 $\pm$ 1.1	9.5–42.9	0.747	0.0001
Body mass index (kg/m ²)	22.9 $\pm$ 0.4	17.1–34.3	0.416	0.0013
Waist to hip ratio	0.941 $\pm$ 0.008	0.79–1.07	0.525	0.0001
Plasma insulin (pmol/l)	50 $\pm$ 8	12–228	0.454	0.0004
Fasting plasma glucose (mmol/l)	7.2 $\pm$ 0.3	3.7–12.8	0.266	0.0457
HbA _{1c} (%)	8.01 $\pm$ 0.27	4.5–12.9	0.249	0.0619
Blood urea nitrogen (mmol/l)	9.2 $\pm$ 0.9	2.8–36.1	0.295	0.0261
Creatinine (μ mol/l)	141 $\pm$ 18	27–716	0.112	0.4074
1/creatinine (l/ μ mol) $\times$ 100	1.27 $\pm$ 0.09	0.139–3.77	–0.197	0.1418
SSBG (mmol/l)	5.02 $\pm$ 0.03	4.44–5.51	0.084	0.5338
SSPI (pmol/l)	691 $\pm$ 29	432–1260	0.449	0.0005
Glucose infusion rate (mg/kg body weight/min)	4.91 $\pm$ 0.28	1.70–11.20	–0.525	0.0001
(mg/kg fat free mass/min)	6.35 $\pm$ 0.33	2.56–13.22	–0.373	0.0046
Insulin sensitivity index (mg/kg body weight/min/(μ U/ml)) $\times$ 100	4.91 $\pm$ 0.42	1.1–16.5	–0.575	0.0001
(mg/kg fat free mass/min/(μ U/ml)) $\times$ 100	6.23 $\pm$ 0.50	1.8–18.2	–0.498	0.0001

Leptin level was log-transformed before correlation analysis. SSBG, steady state blood glucose; SSPI, steady state plasma insulin. Glucose infusion rate and insulin sensitivity index were expressed on the basis of both body weight and fat free mass

level is associated more closely with plasma insulin level than with insulin sensitivity itself; and 3) impaired renal function is another independent factor affecting plasma leptin concentrations.

Subjects and methods

Subjects. Fifty-seven patients with NIDDM (36 males and 21 females) were studied. Eleven patients were treated with diet only, 22 with sulfonylurea, and 24 with insulin. Regarding nephropathy stages [19], 20, 13, 9 and 15 patients had nephropathy stages I (urinary albumin excretion, UAE < 30 mg/24 h), II (UAE, 30–300 mg/24 h), III (UAE > 300 mg/24 h) and IV (plasma creatinine > 180 μ mol/l), respectively. Other clinical characteristics are given in Table 1.

Plasma leptin concentration. Plasma leptin concentration was measured by radio-immunoassay using commercial kits (Human Leptin RIA kit; Linco Research Inc., St. Charles, Mo., USA). The sensitivity of the assay was 0.5 ng/ml.

Clamp study. Euglycaemic hyperinsulinaemic clamp was performed according to DeFronzo et al. [20] using an artificial pancreas model STG 22 (Nikkiso Co., Tokyo, Japan) as previously described [21]. Clamp studies and blood samplings were done after an overnight fast. Evening dose of insulin was discontinued prior to the study. Insulin (Humulin R, Eli Lilly & Company, Indianapolis, Ind., USA) was infused in a primed continuous manner at a rate of 1.25 mU $\cdot$ kg^{–1} $\cdot$ min^{–1}. The

steady state blood glucose level of 5 mmol/l was maintained by adjusting the infusion rate of 20% glucose solution. Blood glucose was monitored at 5-min intervals during the 120-min clamp study. Insulin sensitivity index was defined as glucose infusion rate per body weight or per fat free mass corrected against the steady state plasma insulin level during the last 30 min of the clamps.

Body fat measurement. Body fat mass was measured by dual energy X-ray absorptiometry (model QDR-2000; Hologic, Waltham, Mass., USA), and expressed as percent body fat. Body fat distribution was assessed by waist to hip ratio (WHR).

Other measurements. Glucose was measured by the glucose oxidase method, haemoglobin A_{1c} by high pressure liquid chromatography, and plasma insulin by radioimmunometric assay (Insulin RIABEAD II; Dinabot Co., Tokyo, Japan). Other measurements were by routine clinical methods.

Statistical analysis. Results are expressed as mean $\pm$ SEM. Differences of mean values between two groups were evaluated by Student's *t*-test and difference of medians was assessed by Mann-Whitney's *U*-test. Correlation between two variables was examined by simple regression analysis. Independent associations between one dependent variable and more than two independent variables were assessed by multiple regression analyses. *P*-values less than 0.05 were taken to be statistically significant. Plasma leptin levels were log-transformed before simple and multiple regression analyses, because its distribution was not normal.

Table 2. Multiple regression analysis of factors affecting plasma leptin levels

Independent	Model-1	Model-2	Model-3	Model-4	Model-5	Model-6	Model-7
Percent body fat	0.747 ^a	0.655 ^a	0.435 ^a	0.380 ^a	0.408 ^a	0.405 ^a	0.429 ^a
Waist to hip ratio		0.313 ^a	0.401 ^a	0.333 ^a	0.279 ^a	0.215 ^b	0.231 ^b
Gender			-0.319 ^b	-0.297 ^b	-0.366 ^a	-0.317 ^a	-0.386 ^a
Insulin sensitivity				-0.218 ^c	-0.160 ^c	-0.103 (NS)	
1/creatinine					-0.259 ^a	-0.263 ^a	-0.282 ^a
Basal insulin level						0.198 ^b	0.225 ^b
<i>R</i> ²	0.558 ^a	0.656 ^a	0.713 ^a	0.749 ^a	0.803 ^a	0.832 ^a	0.825 ^a

Standard regression coefficients (β) were given with the level of significance. ^a $p < 0.001$; ^b $p < 0.01$; ^c $p < 0.05$. NS, Not significant. *R*², multiple coefficient of determination. Dummy variables were used for gender (female = 1, male = 2). Insulin sen-

sitivity indicates that of body weight basis. When the fat free mass-based insulin sensitivity was entered in the models, essentially the same results were obtained

Results

Plasma leptin level in the 57 NIDDM patients was 6.1 ± 0.8 ng/ml (mean $\pm$ SEM). Female patients had significantly higher leptin concentrations than male patients (3.9 ± 0.6 vs 9.7 ± 1.5 ng/ml, $P = 0.0001$ by Student's *t*-test or by Mann-Whitney's *U*-test).

Table 1 shows results of simple regression analyses between leptin and other variables. Plasma leptin level correlated positively with percentage body fat, BMI, WHR and fasting insulin, whereas it correlated inversely with insulin sensitivity index. No significant correlation was found between leptin and renal function by simple regression analyses.

Table 2 summarizes multiple regression analyses of factors independently affecting plasma leptin levels. The model-1 through model-3 indicated percentage body fat, WHR, and gender were all significant and independent determinants of plasma leptin levels. When insulin sensitivity and renal function were included as the 4th and 5th variables, respectively, the association between leptin and these variables appeared to be significant (model-4 and 5). However, in the model in which plasma insulin level was considered (model-6), the association between insulin sensitivity and leptin disappeared whereas the impact of insulin level on leptin became significant. In this model, the inverse association between renal function and leptin levels remained significant, indicating the association was independent of insulin sensitivity and fasting plasma insulin level. Finally, more than 82 % of the variation in plasma leptin concentration was explained by five variables including total body fat mass, fat distribution, insulin level, gender and renal function (model-7).

Discussion

Plasma leptin levels correlate with body fat mass [7–9]. Leptin level may be influenced by other factors including renal function and insulin resistance [13–15]. Results of the present study indicated renal function

as an important determinant of plasma leptin levels. Insulin resistance appeared to contribute to hyperleptinaemia by raising plasma insulin levels.

It is a novel finding that renal function was significantly associated with plasma leptin levels. We had anticipated this because renal failure results in insulin resistance and hyperinsulinaemia [17]. However, the association between renal function and plasma leptin level was independent of plasma insulin and insulin resistance. Therefore, other explanations are needed. The leptin receptors have a wide distribution among organs including the central nervous system and the kidney [18], suggesting that the kidney may play a role in the clearance of plasma leptin. According to a preliminary report by Coyne et al. [22], plasma leptin level in haemodialysis patients is elevated and not because of accumulation of leptin degradation products. They did not address the possible effect of hyperinsulinaemia on leptin. We demonstrated that elevation in plasma leptin due to impaired renal function occurred independently of other metabolic and endocrinological factors. The kidney may play a physiological role in leptin clearance [23]. If the intact leptin molecules are accumulated due to renal failure, hyperleptinaemia might account, at least in part, for malnutrition of patients with chronic renal failure.

The effects of insulin on plasma leptin level are controversial. Exogenously administered insulin increases the *ob*-gene expression and secretion of leptin in rodents [24]. In humans, insulin infusion for 3 h was reported not to increase plasma leptin levels [13]. Other studies [11, 12] reported an increased plasma leptin level after a 6–8 h hyperinsulinaemic clamp. In the present study, we revealed the significant impact of fasting plasma insulin concentration on plasma leptin levels in patients with NIDDM. Taken together, these studies suggest that plasma leptin level in humans is regulated by insulin not in an acute but in a chronic manner.

It has not been established whether insulin resistance affects plasma leptin levels independently. Segal et al. [13] showed that insulin-resistant lean men had higher plasma leptin levels than insulin-sensitive

lean men, and concluded that insulin resistance is associated with elevated leptin level independently of body fat. In that study, however, a higher plasma insulin level in the insulin resistant men was not taken into account. In contrast, Dua et al. [14] and Larsson et al. [15] failed to detect a significant relationship between plasma leptin concentration and insulin-sensitivity assessed by Bergman's minimal model [14] or by euglycaemic hyperinsulinaemic clamp technique [15]. Simple regression analysis in our study showed a significant inverse correlation between plasma leptin level and insulin sensitivity index by the euglycaemic hyperinsulinaemic clamp technique. However, multiple regression analyses did not indicate insulin sensitivity as a significant independent factor for plasma leptin concentrations when plasma insulin level was included in the analysis. Although these results could not deny the independent effect of insulin resistance on hyperleptinaemia, we interpret the data to indicate that insulin resistance indirectly contributes to hyperleptinaemia by raising plasma insulin levels.

In conclusion, our results suggested that plasma leptin level was regulated or affected by multiple factors including renal function. Insulin resistance appeared to affect plasma leptin level via hyperinsulinaemia, although insulin sensitivity itself was not shown to be an independent determinant of leptin. Further studies are needed to clarify the precise mechanisms for these correlations, especially the relationship between renal function and leptin, to better understand obesity, malnutrition and related metabolic disorders.

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