

CLINICAL STUDY

Role of diabetes in influencing leptin concentration in elderly overweight patients

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Abstract

Background: Leptin, the product of the *ob* gene, could have a significant role in the pathogenesis of obesity and non-insulin-dependent diabetes mellitus. However, it is still debated whether different degrees of glucose tolerance may affect plasma leptin concentrations in obese patients.

Objective: To investigate whether diabetes might influence leptin concentrations in obese patients.

Methods: We evaluated clinical parameters, anthropometric measures, and sex hormones, fasting plasma leptin, glucose and insulin concentrations in 100 elderly obese diabetic patients and 100 obese non-diabetic control individuals matched for age and sex.

Results: After adjustment for age and fat mass, plasma leptin concentrations did not differ between diabetic and non-diabetic obese individuals, in both men and women. In all patients leptin was significantly related to body mass index, fat mass and the homeostasis model insulin resistance index; moreover we observed a significant relationship with fasting plasma glucose and age in diabetic obese women, and with blood pressure values and testosterone concentrations in diabetic obese men. Multiple regression analysis revealed age and fasting plasma glucose to be the only independent determinants of fasting plasma leptin in diabetic obese women.

Conclusions: These data suggest that leptin concentrations do not differ between obese diabetic and obese non-diabetic elderly patients. Among correlates of the metabolic syndrome, systolic pressure seems to be related to leptin only in men. In the postmenopausal or andropausal status, sex hormones are related to leptin concentrations only in diabetic men; in diabetic women, however, high glucose seems to be relevant in maintaining the same leptin concentrations as in non-diabetic women with similar degree of obesity.

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Introduction

The discovery of leptin, the product of the obesity (*ob*) gene, as a hormone secreted by adipocytes has been a major advance in understanding the regulation of energy balance (1). In *ob/ob* mice obesity is caused by the absent or extremely reduced production of leptin by the adipose tissues (1, 2). Administration of the *ob* gene product to these animals leads to weight loss, ameliorates glucose intolerance and improves insulin sensitivity (1). In humans, leptin concentrations are directly proportional to body fat mass, suggesting that obese individuals might be resistant to the leptin signal, rather than leptin deficient, though the pathogenetic mechanism remains unknown (3). Leptin concentrations, however, vary widely among individuals with similar amounts of adipose tissue, indicating a possible influence of other inherited/environmental factors in

determining plasma hormone concentrations (3, 4). Greater concentrations of leptin have been reported in women compared with those in men, even when individuals of similar total fat mass were considered (5, 6), suggesting a role of sex hormones in influencing its concentration. Testosterone has been found to decrease adipocyte production of leptin (7); moreover, leptin concentrations have been shown to correlate with insulin concentration and the degree of insulin sensitivity, and a role for leptin has been proposed in the aetiology of insulin resistance and non-insulin-dependent diabetes mellitus (NIDDM) (8–10).

Whether leptin concentrations are different in normoglycaemic individuals and patients with NIDDM is still questioned, as previous studies have yielded contrasting results (11–13). Even less is known concerning the possible different effects of metabolic variables on leptin in obese diabetic patients compared

with those individuals with normal glucose tolerance but a similar degree of obesity, particularly in the elderly. Therefore, we compared the relationship between fasting leptin concentrations and other clinical and metabolic characteristics in two groups of ageing overweight patients with and without NIDDM.

Participants and methods

Participants

We consecutively recruited 100 elderly (aged more than 55 years), overweight or obese (body mass index (BMI) $>27 \text{ kg/m}^2$) patients with NIDDM and 100 non-diabetic obese control individuals comparable for age, sex and degree of obesity. All these patients attended the outpatient clinic for Metabolic Diseases at the University of Ferrara. Exclusion criteria were the coexistence of any other serious illness and high blood pressure values ($>150/90 \text{ mmHg}$ or current antihypertensive therapy). NIDDM was defined as non-ketosis-prone diabetes by medical history and current treatment with diet or oral agents. Administration of insulin for glycaemic control was considered an exclusion criterion. In controls, diabetes was excluded by an oral glucose tolerance test. The local medical ethics committee approved the study and informed consent was obtained from all participants.

Anthropometric measurements

After an overnight fast, all participants were admitted to the outpatient clinic, weighed in light clothing, their heights were recorded and their BMI calculated. Waist circumference was measured between the lower rib and the iliac crest, at the end of a normal expiration. Body composition was determined by bioelectrical impedance using a BIA 109 instrument (RJL System, Detroit, MI, USA) with the individual in the supine position.

Biochemical analysis

A blood sample was drawn for determination of fasting plasma glucose (by the glucose oxidase method),

plasma insulin (chemiluminescent sandwich assay with no cross-reactivity with proinsulin; Access System, Beckman, Palo Alto, CA, USA) and leptin concentration (radioimmunoassay; Linco Research, St Charles, MO, USA). Glycated haemoglobin (HbA_{1c}) was determined by HPLC. Radioimmunoassay commercial kits were used to determine total testosterone, dehydroepiandrosterone sulphate (DHEAs) and sex hormone-binding globulin (SHBG). Both intra-assay and interassay coefficients of variation in all of the above methods were less than 5%. 17β -Oestradiol was assayed by ELISA. The homeostasis model insulin resistance index (IRI) (HOMA index), suitable as a simple assessment of insulin sensitivity, was calculated using the formula $[\text{fasting plasma glucose (mmol/l)} \times \text{fasting IRI } (\mu\text{U/ml})]/405$ (14) and the mean indexes of the groups were compared.

Statistical analysis

Data were stored and analysed using SPSS 7.5 package (SPSS, Evanston, IL, USA) for Windows. Biochemical parameters not normally distributed were analysed after being logarithmically transformed. Differences between groups were compared by Student's unpaired *t*-test, one-way ANOVA or ANCOVA. Simple and partial correlation coefficients between the variables were determined and multiple regression analysis was performed to determine relationships between variables of interest. Data are expressed as mean \pm s.d. or median (range); statistical significance was accepted at $P < 0.05$.

Results

Clinical and metabolic characteristics of the four groups are shown in Tables 1 and 2. Diabetic obese patients had waist:hip ratio, BMI and systolic blood pressure values similar to those of obese non-diabetic individuals. Fasting plasma glucose and HbA_{1c} were greater in diabetic obese women and in diabetic obese men than in obese women and obese men by inclusion criteria. Plasma insulin concentrations were similar in the four groups.

Table 1 Clinical characteristics of the four study groups.

Characteristic	DOM (<i>n</i> = 49)	OM (<i>n</i> = 51)	DOW (<i>n</i> = 48)	OW (<i>n</i> = 52)
Age (years)	64 \pm 7	61 \pm 9	64 \pm 8	64 \pm 8
Diabetes duration (years)	8 \pm 6	–	7 \pm 5	–
Systolic blood pressure (mmHg)	138 \pm 11	134 \pm 14	137 \pm 13	132 \pm 15
Diastolic blood pressure (mmHg)	80 \pm 5	78 \pm 6	81 \pm 4	80 \pm 4
BMI (kg/m^2)	30.4 \pm 4.5	29.2 \pm 2.4	32.7 \pm 4.4	31.4 \pm 3.5
Waist:hip ratio	1.005 \pm 0.071	0.978 \pm 0.080	0.931 \pm 0.078	0.901 \pm 0.080
Fat mass (kg)	26.6 \pm 10.6	24.6 \pm 8.4	31.1 \pm 6.4	28.7 \pm 5.9

DOM, DOW, diabetic obese men or women; OM, OW, non-diabetic obese men or women.

Table 2 Metabolic and hormonal parameters of the four study groups. Data are expressed as mean±s.d. or median (range). Abbreviations as in Table 1.

Parameter	DOM (n = 49)	OM (n = 51)	DOW (n = 48)	OW (n = 52)
Plasma glucose (mmol/l)	9.77±3.1***	5.55±0.5	9.88±2.9***	5.38±0.5
Plasma insulin (µU/ml)	7.55 (2.7–20.0)	7.0 (3.2–34.0)	7.0 (2.0–45.0)	7.2 (3.0–87.0)
HbA _{1c} (%)	8.6±1.2***	5.4±0.8	8.5±1.5***	5.3±0.9
HOMA index	3.2 (1.35–15.01)***	1.61 (0.7–6.97)	3.48 (0.91–13.29)*	1.72 (0.64–18.69)
17β-Oestradiol (pg/ml)	–	–	4.7±10.1	9.6±17.0
Testosterone (ng/ml)	3.34±1.27	3.71±1.14	–	–
SHBG (nmol/l)	36.2±24.5	35.3±24.2	41.5±23.0	54.9±33.6
DHEAS (µg/dl)	116.2±80.0	148.8±88.0	87.7±78.9	75.5±56.6

* $P < 0.05$ compared with group OW; *** $P < 0.001$ compared with non-diabetic group of same sex.

Biochemical and hormonal parameters of the four study groups are reported in Table 2. We did not observe differences in plasma hormone concentrations between obese and diabetic obese patients, either in men or in women.

Analysis of covariance for age, fat mass, glucose and insulin, oestradiol or testosterone, revealed that both leptin concentrations (22.6 ± 1.5 ng/ml in diabetic obese women, 20.2 ± 1.3 ng/ml in non-diabetic obese women, 8.5 ± 0.5 ng/ml in diabetic obese men and 8.9 ± 0.8 ng/ml in non-diabetic obese men; all $P = \text{NS}$) and leptin:fat mass ratio (0.741 ± 0.05 in diabetic obese women, 0.691 ± 0.06 in non-diabetic obese women, 0.315 ± 0.02 in non-diabetic obese men and 0.307 ± 0.03 in diabetic obese men; all $P = \text{NS}$) were significantly greater in women than in men; no difference was observed between diabetic and non-diabetic patients of either sex.

Univariate analysis (Table 3) revealed that leptin concentrations were positively related to BMI, fat mass, insulin and HOMA index in all groups. In women, no relationship was apparent with any hormonal parameter, whereas in diabetic obese men a negative relationship with testosterone was observed. No relation was observed between DHEAS and leptin in any of the groups. Moreover, in men we observed an interesting direct relationship between leptin and both systolic and diastolic blood pressures, irrespective of the presence of diabetes, whereas in diabetic obese

women leptin was associated with fasting plasma glucose, but not with HbA_{1c}. Some of these correlations in diabetic patients are depicted in Fig. 1.

To address further the possible differences in the relationship between serum leptin concentration and other variables among the study groups, multiple regression analysis was applied. Using this analysis (Table 4), we observed a strong effect of age and fasting plasma glucose on serum leptin ($P < 0.001$ and $P = 0.023$, respectively) in diabetic obese women, whereas in men BMI and fat mass were the only parameters significantly contributing to leptin concentrations.

Discussion

The main result of the present study is that, even though leptin concentrations do not differ between obese and diabetic obese patients in either men or women, some correlates of the so-called metabolic syndrome are associated with this parameter in the two clinical conditions. The intrinsic nature of a cross-sectional study like this one, however, does not allow one to draw conclusions on the causal relationship between leptin and other variables; the relatively small sample size also prevents such conclusions.

In our study population, diabetic obese individuals had a slightly, but not significantly, greater fat mass compared with non-diabetic obese participants; no

Table 3 Spearman's coefficients of the relationship between leptin (log-transformed values) and other variables in the four study groups. Numbers within parentheses are P values; those in bold are statistically significant.

Variable	DOM (n = 49)	OM (n = 51)	DOW (n = 48)	OW (n = 52)
Age	–0.117 (0.243)	–0.223 (0.165)	0.414 (0.007)	–0.053 (0.403)
BMI	0.711 (0.000)	0.527 (0.007)	0.412 (0.007)	0.410 (0.023)
Fat mass	0.766 (0.000)	0.399 (0.037)	0.421 (0.006)	0.616 (0.001)
Plasma insulin†	0.350 (0.016)	0.515 (0.008)	0.331 (0.026)	0.406 (0.024)
HOMA index†	0.321 (0.049)	0.519 (0.008)	0.207 (0.116)	0.389 (0.030)
DHEAS	–0.148 (0.188)	–0.090 (0.353)	–0.010 (0.476)	–0.127 (0.215)
SHBG	–0.232 (0.081)	0.242 (0.152)	–0.180 (0.150)	–0.196 (0.277)

†Log-transformed values.

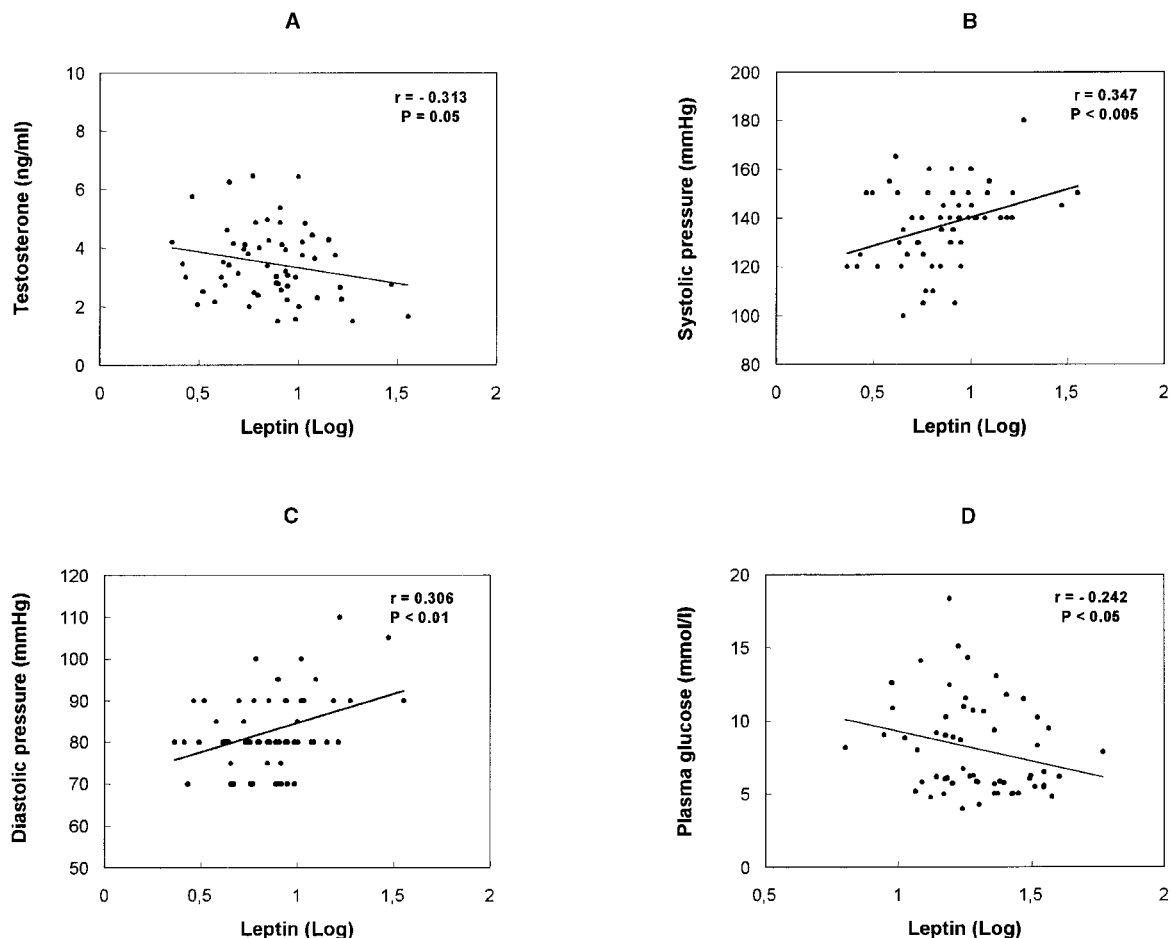


Figure 1 Plots of plasma leptin concentrations and some correlates of the metabolic syndrome in diabetic patients: (A) testosterone in men; (B) systolic blood pressure in men; (C) diastolic blood pressure in men; (D) fasting plasma glucose in women.

Table 4 Multiple regression analysis of the relation between leptin concentrations and variables of interest.

	Diabetic obese individuals		Non-diabetic obese individuals	
	Standard coefficient	P value	Standard coefficient	P value
Men				
Age	0.028	0.795	-0.007	0.978
Systolic blood pressure	-0.065	0.610	0.013	0.962
Diastolic blood pressure	0.088	0.472	-0.030	0.899
BMI	0.497	0.010	0.374	0.315
Fat mass	0.374	0.035	0.294	0.369
HOMA model	0.033	0.760	0.389	0.052
Testosterone	-0.070	0.504	-0.149	0.558
Regression coefficient	R^2 0.729; $P < 0.001$		R^2 0.696; $P < 0.02$	
Women				
Age	0.668	<0.001	-0.019	0.403
BMI	0.195	0.251	-0.347	0.306
Fat mass	0.213	0.198	0.867	0.013
Plasma glucose	-0.347	0.007	-0.240	0.203
HOMA model	0.409	0.008	0.332	0.058
Regression coefficient	R^2 0.634; $P < 0.001$		R^2 0.554; $P < 0.0016$	

significant difference was detected, however, when mean values of fasting leptin concentration were compared, even after adjustment for confounding factors. This partially confirms the findings of previous studies in populations of different ethnicity (11, 13). However, in women we confirmed the presence of greater leptin concentrations in women, independent of body fat mass (5, 6) and waist circumference, an index of truncal obesity.

Many reasons have been invoked to explain this discrepancy between the sexes. Increased leptin production has been reported in peripheral-subcutaneous than in abdominal-visceral fat depots (15); as women have a predominantly subcutaneous distribution of fat, the presence of a fat depot that produces more leptin mRNA than adipose cells from other sites (16) has been proposed. Alternatively, the difference could be due to the steroid milieu, as proposed by several authors (17, 18). Increasing evidence suggests that testosterone decreases the circulating concentrations of leptin, probably by direct inhibition of leptin expression in the adipocyte (19); in contrast, the relationship between circulating oestrogens and leptin remains inconclusive. Some studies have not shown an influence of oestrogens (18, 20), whereas others did so (21, 22). A recent report by Elbers *et al.* (23) described a slight but significant increase in serum leptin concentrations after a 2-month period of oestrogen replacement therapy in healthy postmenopausal women, whereas this effect was not evident in a placebo group. In our ageing women, low concentrations of 17 β -oestradiol were unable to affect leptin concentrations significantly; with regard to testosterone, even though we did not observe significant differences between diabetic and non-diabetic obese men, confirming previous observations of a blunted effect of testosterone as a major determinant of serum leptin in men (24, 25), an inverse relationship of testosterone and serum leptin was observed in diabetic men only. Previous authors have described this relationship (23, 26); our data offer a further contribution to the connection between diabetes and obesity, showing that, in men with the same degree of obesity and the same blood pressure values, testosterone is related to leptin only in diabetic patients. In none of the groups were we able to observe a relationship with DHEAS, excluding an effect of this hormone on leptin concentration in elderly individuals also, as already pointed out in obese children and adolescents (23).

Both plasma insulin concentrations and indexes of insulin resistance have been associated with serum leptin independently of variance in total or percentage body fat mass (27); there is, however, evidence that plasma leptin concentrations vary much more as a function of the circulating insulin concentrations than of the degree of insulin resistance itself (28). Long-term exogenous hyperinsulinaemia leads to an increase in *ob* gene expression and, consecutively, in plasma leptin

concentrations in both humans and animal models (29, 30). The relationship between the two hormones seems to be reciprocal: Seufert *et al.* (10) recently reported that the application of leptin to freshly obtained human islets resulted in a marked suppression of both insulin secretion and the expression of proinsulin mRNA within the islets, emphasising the presence of a negative feed-back loop between pancreatic β -cell insulin secretion and adipocyte leptin synthesis, which could be involved in the pathogenesis of the so-called adipogenic NIDDM (31, 32). However, studies performed in humans have yielded contrasting results concerning the existence of an inhibitory effect of leptin on insulin synthesis and secretion, at least in non-diabetic individuals (33). We did not observe differences in either leptin or plasma insulin concentrations between obese and diabetic obese patients of either sex; the obese condition was probably not able to reveal any reciprocal regulatory effect of the two hormones, at least for fasting insulin concentrations within the normal range, even though a direct relationship between insulin and leptin was confirmed in our four groups of patients. It is possible, however, that measurement of post-prandial insulinaemia, not available in the present study, would have reinforced this relationship, which is much more influenced by the day-long insulin response to meals, as shown in previous studies performed in non-diabetic individuals (34).

A recent report by Panarotto *et al.* (35) has described lower leptin concentrations in women with diabetes or impaired glucose tolerance, compared with those in controls. Our data only appear to be in contrast with these findings; our patients had, actually, a greater degree of obesity than those studied by Panarotto's group, and greater fasting plasma glucose concentrations. It is interesting to note that, in both studies, leptin was inversely related to the degree of hyperglycaemia in diabetic women, irrespective of plasma hormone concentrations. These findings suggest that, in women with NIDDM, the synthesis of leptin by adipose tissue is susceptible to *in vivo* regulation by both glucose and insulin, raising the possibility that a relative deficiency of leptin could be associated with an increased adiposity and a worse metabolic control in obese individuals with NIDDM. Our two subgroups of diabetic patients had absolutely superimposable HbA_{1c} values, and so we could not detect this difference in our study, but it has been shown that poorly controlled NIDDM was accompanied by a significant reduction in leptin concentrations in morbidly obese individuals (13). The results of our multiple regression analysis, showing glucose as independent predictor of leptin in these patients, confirm its regulatory role of hormone concentrations in NIDDM.

An association between systolic blood pressure and serum leptin concentrations in hypertensive men has been described (36). Among other mechanisms, sex

hormones probably have a role in mediating these sex differences: in spontaneously hypertensive rats, it has been shown that early treatment with a testosterone antagonist attenuates the development of high blood pressure (37); moreover, there is a large nocturnal increase in circulating leptin concentrations, and an influence of sex on the relationship between nocturnal leptin increment and blood pressure regulation may be supposed. Our observations confirm this link between leptin and blood pressure values in normotensive patients also; interestingly, the correlation is maintained in diabetic patients despite the presence of insulin concentrations similar to those in non-diabetic individuals, suggesting that other elements besides the sympathetic effect of insulin could link leptin to blood pressure regulation in NIDDM. A recent report by Schorr *et al.* (38) supports the hypothesis that adipose mass is an important determinant of blood pressure in young normotensive non-obese men; our results show for the first time that this relation holds also for older and obese individuals, further underlining the physiological connection between leptin and other well-established correlates of the insulin resistance syndrome.

In summary, the results of the present study suggest that, in overweight to morbidly obese individuals, the presence of diabetes does not influence fasting leptin concentrations. Women have increased plasma leptin concentrations compared with fat-mass-matched men, and this was true in both diabetic patients and normal individuals; hyperglycaemia, however, seems to have a major role in influencing leptin concentrations in diabetic patients. Whether these differences might play a part in maintaining obesity or even in the pathogenesis of NIDDM requires further investigation.

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