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Leptin Levels in Diabetic and Nondiabetic Subjects

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The role of leptin in human pathophysiology elicits considerable interest in view of its potential role as a treatment tool for obesity and other insulin resistant states, like type 2 diabetes mellitus (T2DM). Leptin has been extensively studied in obese humans, and much less so in other pathologic conditions. Leptin level has been reported to correlate with percent body fat mass (%FM), fasting serum insulin (FPI), insulin sensitivity and blood pressure. The aim of this study was to compare the leptin concentration, and its relationship with some anthropometric and biochemical parameters related to insulin resistance in 140 moderately obese type 2 diabetics (T2DM) and 160 age and weight matched non-diabetic controls in order to get a better insight into the possible role of leptin in the metabolic abnormalities of diabetes. The leptin levels were lower in the diabetic population only when both sexes were combined ($p < 0.05$) and were higher in the females of both groups. Among the nondiabetics, the leptin levels appeared to be related to BMI, %FM, HDL and FPI, while this was not the case in the diabetics. After correction for BMI, leptin appeared to be correlated with the FPI levels only in the non-diabetic females. When plasma leptin was included in a multiple linear regression model with plasma leptin as a dependent variable, BMI, W:Hr and FPI levels were significantly related to leptin in the non diabetic population, while no relationship reached the level of statistical significance among the diabetics, with the exception of the borderline value for the FPI ($p = .052$). In conclusion, leptin levels were independent of any of the parameters examined in our diabetic population, possibly due to the progressive loss of the normal mechanisms of leptin regulation with advancing disease. Conclusive data can only be obtained from the longitudinal study of a cohort of newly diagnosed diabetic subjects.

Key Words: Leptin; obesity; diabetes mellitus.

Introduction

The role of leptin in human pathophysiology elicits considerable interest in view of its potential role as a treatment tool for obesity and other insulin resistant states *(1)*.

Leptin has been extensively studied in obese humans, but much less so in other pathologic conditions. The levels of this hormone increase with increasing fat mass *(2,3)*, and the relationship is stronger when the increase is predominantly in visceral fat *(3)*. The circulating levels seem to be modulated by fasting and refeeding *(4,5)*. Cigarette smoking apparently decreases leptin *(6)*, and many drugs have been associated with changing leptin levels *(7)*, notably insulin in prolonged infusion *(8)*, whereas acutely administered insulin did not seem to affect leptin *(9)*. It has been reported that leptin levels correlate with fasting serum insulin (FPI), insulin sensitivity and blood pressure *(10)*.

The aim of this study was to compare the leptin concentrations and its relationship with some anthropometric and blood parameters related to insulin resistance in a population of moderately obese type 2 diabetics (T2DM) and age–weight matched non diabetic controls in order to get a better insight into the possible role of leptin in the metabolic abnormalities of diabetes.

Materials and Methods

We cross-sectionally examined the leptin levels in a group of 140 presenile to senile obese diabetic (T2DM) subjects and 160 matched non diabetic individuals (see Table 1 for age details). All the subjects gave their informed consent. The study was approved by the ethics committee.

The anthropometric and biochemical data were obtained under fasting conditions from 8:00 to 9:00 AM. The weight was obtained after the subjects removed their shoes and outer garments and BMI was calculated according to the standard formula: Kg/m^2. Waist and hip circumferences were taken according to standard procedures ("waist" was measured at the point of maximum protrusion above or below 4 cm from the umbilicus; "hip" at the line bridging the two greater trochanters) and the ratio of the two (W:Hr) was recorded. Blood pressure was measured three times after 5 minutes' rest in a comfortable environment at 3 minute

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intervals by the standard Riva Rocci sphygmomanometer method and the mean of the three measurements was recorded in the patient's file. All the subjects were on their usual diet. The obese subjects were on no medication, apart from antihypertensive drugs that were discontinued 2 days prior to the examination. All the females were postmenopausal. The diabetic subjects were on a normocaloric diet or diet+sulphonilurea therapy. Those treated with insulin or metformin were excluded. All the heavy smokers (>5 cigarettes/day) were excluded; all light smokers, whose distribution among the nondiabetic and diabetic groups was similar (26% and 18%, respectively), were asked to refrain from smoking for at least 12 h. The blood was withdrawn and examined immediately at the local laboratory for the common biochemical parameters, including total cholesterol (Chol), HDL-cholesterol (HDL), triglycerides (TGL); only in T2DM, glycohemoglobin (HbA1c) was also measured immediately by HPLC to monitor the level of metabolic control during the weeks before the study. Serum obtained by centrifugation was also aliquoted and stored frozen at –20°C until analyzed in duplicate at the INRCA Hormone Research Laboratory with a RIA for FPI (DPC, CA, USA; intraassay c.v. 5.8%, interassay c.v. 8.9%) and IRMA for leptin (DSL, TX, USA, intraassay c.v. 3.9%; interassay c.v. 5.7%). The mean HbA1c in this population was 6.9 ± .25. All the subjects were in good metabolic control. None of them had macroalbuminuria (microalbumin 2.4–35 mg/min).

To evaluate the insulin sensitivity and the insulin secretion we calculated the %b, an index of insulin secretion, and the %S, an index of insulin sensitivity, according to the Homeostatic Model Assessment *(9)*.

As our and other groups already showed leptin increases with body fat in non-diabetic subjects with a very high significance *(2,3,10)*. We also measured percent fat mass (%FM) by multifrequency bioimpedance in all T2DM patients and in a smaller sample from our non-diabetic population chosen according to an age/BMI case-control design (M:F = 8:8), in order to assess whether the positive correlation with leptin levels might be disrupted by the diabetic condition.

Age, sex distribution, and BMI are reported in Table 1 for the two groups. Diabetes duration and HbA1c levels are listed for the diabetic group only.

Statistical analysis was performed by SPSS (Chicago, ILL, USA): the least squares method was used for descriptive statistics on raw or logtransformed (leptin, insulin, %S and %β) data according to skewness. When a non-normal distribution was found, as expected for some original data sets (i.e. those of insulin and W:Hr), the data were log transformed, and then used for subsequent statistical analyses, although the non-logtransformed mean are reported in the tables. The least statistical significance level was set at a level of $p < 0.05$.

Table 1
Comparison of Variables (means ± SD)

	Non Diabetic	Diabetic	*p*	units
Age	62 ± 7	66 ± 9	NS	yrs
BMI	34.1 ± 2	35.2 ± 4	NS	Kg/m^2
Smokers	22%	18%		–
M:F	48:52	43:57		–
Duration of disease		16 ± 8		yrs
W:Hratio*	.90 (±1.2)	.92 (±1)	NS	–
HbA1c		6.9 ± .25		–
SBP	145 ± 10	158 ± 18	NS	mmHg
DBP	88 ± 5	89 ± 4	NS	mmHg
Chol	191 ± 42	219 ± 46	$p = .000$	mg/dL
Trigl	120 ± 74	144 ± 85	$p = .07$	mg/dL
HDL	49 ± 16	57 ± 14	$p = .003$	mg/dL
FPI*	12.7 (±8)	9.1 (±10)	p = NS	mU/L
Leptin*	28.4 (±19)	19.6 (±4)	$p = .049$	ng/mL
HOMA %S*	35 (±15)	22 (±22)	p = NS	–
Homa %β*	180 (±30)	98 (±28)	$p = .05$	–
% FM	38 ± 3**	38 ± 2	p = NS	

*Due to the non normal distribution, the SD is given in brackets; **$n = 16$.

Table 2
Leptin Concentrations in the Subgroups of Patients According to Sex and Diagnosis of Diabetes (means ± DS). Log-transformed Data were Used for Subsequent Analyses

	Non Diabetic	Diabetic	*p*
F	30.6 ± 19	25.3 ± 44	NS
M	19.4 ± 11	12.9 ± 35	NS
p =	.000	NS	

Results

The diabetic and nondiabetic groups were similar at baseline with the obvious exception of the lipids, and the %β (see Table 1). The leptin levels were lower in the diabetic population when both sexes were combined, with a $p = 0.049$; when the population was subdivided according to sex, such difference disappeared. On the other hand, the leptin levels remain higher in the females of both groups, but the gender difference reached the level of statistical significance only in the non-diabetic population (Table 2).

Table 3 shows the correlation of the study variables in the two populations. Among the nondiabetics the leptin levels appeared to be related to BMI, HDL and FPI, while this was not the case in the diabetics (Table 3).

After correction for BMI, leptin appeared to be correlated with the FPI levels and the HOMA %β in non-diabetic females. This correlation did not hold true in diabetic females or in either male group (Table 4).

Table 3
Correlations of Leptin in Both Groups

	Non Diabetic	Diabetic
Age	p = NS	p = NS
BMI	.000	p = NS
W:Hratio	.001	p = NS
SBP	p = NS	p = NS
DBP	p = NS	p = NS
Chol	p = NS	p = NS
Trigl	p = NS	p = NS
HDL-Chol	.04	p = NS
FPI	.05	p = NS

Table 4
Correlations of Leptin
in the 2 Sexes After Correction for %FM and BMI

	F, diabetic	F, non diabetic	M, diabetic	M, non diabetic
Insulin	p = NS	p < .05	p = NS	p = NS
HOMA %β	p = NS	p < .05	p = NS	p = NS
%FM	p = NS	p < .01	p = NS	p < .01

Table 5
Multiple Linear Regression
with Plasma Leptin as a Dependent Variable

Independent variable	Non diabetics	Diabetics
Age	p = NS	p = NS
BMI	p < .002	p = NS
W:H	p < .05	p = NS
Insulin	p < .05	p = .052
Blood glucose	p = NS	p = NS
HbA1c		p = NS
R^2	54%	36%

When plasma leptin was included in a multiple linear regression model with plasma leptin as a dependent variable (Table 5), BMI, W:Hr, and FPI levels remained significantly related to leptin in the non-diabetic population, while no relationship reached the level of statistical significance among the diabetics, with the exception of the borderline value for the FPI (p = .052).

Finally, our data also demonstrated a different behavior between the non-diabetic and the diabetic subjects with respect to the relationship between leptin and %FM. In the former group, the leptin levels appeared to be related to the overall prevalence of the body fat and its distribution, while this relationship did not hold true in the diabetics.

Discussion

The most relevant result of this study is that leptin levels are not dependent on any of the parameters examined in the diabetic population. These relationships are still matter of debate in the literature. Opposite to what has been recently demonstrated in a large population of nondiabetic subjects by our group *(13)*, some authors *(14)* did not find an age dependency of leptin in diabetics. On the other hand Fox et al. demonstrated *(15)* a weak effect of age in 168 diabetic subjects. However, these subjects were remarkably younger than ours (46.8 ± 12.7 vs 66 ± 9 y), and although not stated, they probably had a shorter duration of disease. As in our study, the leptin levels were lower in the diabetic population, but in the experience of these authors, after adjusting for body fat and age, the difference reached statistical significance (<0.002). As in our study, sex and the W:Hr were not significantly related to the leptin concentration.

Adami et al. carried out a study in 112 subjects, and found that only in females were the BMI and the serum leptin concentrations highly associated and fitted an exponential model, while in males, the relationship was a weaker one *(16)*. These subjects were also younger, with a mean age of 33 yr. Thus, the fact that BMI correlated with leptin in our nondiabetic women, but not in their diabetic counterpart has to be taken into serious account. On the other hand, the low number of men included in this study might explain why we cannot find any statistically significant correlation between leptin and BMI in the non-diabetic male subgroup.

Haffner et al. *(17)* demonstrated that the leptin concentrations were not different in diabetics and non-diabetics and that the association of leptin with the components of obesity, principally the BMI, was similar in diabetic and nondiabetic subjects. These conclusions, despite being in apparent contrast to our data, when set in the proper context rather lend support to ours. The subjects in the San Antonio study, like in the study reported formerly *(15)*, were younger (48 ± 1 yr vs 66 ± 9). The subjects were recruited during the second phase of The San Antonio Heart Study started in 1991. During this phase of the study conducted for 7 years, the diagnosis of diabetes mellitus was made with a 75-g OGTT, and leptin was measured at the same time. Thus, also Haffner's cohort was notably represented by newly diagnosed subjects.

In our subjects, the association with insulin was of borderline statistical significance, while insulin has been shown to increase plasma leptin concentration *(18)*: this seems to indicate that the link between leptin and other metabolic parameters becomes progressively looser as the duration of the disease increases.

Overall, the studies discussed above *(15–18)* examined younger subjects in an earlier stage of the disease. The con-

sistent existence of a correlation between leptin and some of the biological and biochemical variables under study in these subjects at the beginning of the disease, and the absence in ours, who were at a more advanced stage, points to the progressive loss of the normal mechanisms of leptin regulation with advancing disease. Conclusive data can only be obtained from the longitudinal study of a cohort of newly diagnosed diabetic subjects

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