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Leptin concentrations do not correlate with fat mass nor with metabolic risk factors in morbidly obese females

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ABSTRACT. Aims: To investigate the determinants of leptinemia in a cohort of morbid obese females compared to those of normal weight and mild-to-moderate obesity, and the relationships between leptin and metabolic derangements associated with obesity. *Methods:* Recruited females were: moderately obese [n=44; body mass index (BMI) 25-40 kg/m^2], morbidly obese (n=34; BMI ≥40 kg/m^2) and normal weight volunteers (n=12; BMI 19-25 kg/m^2). Fat mass assessed by bioelectrical impedance and fat distribution by waist-to-hip ratio (WHR) were determined in all subjects. Biochemical determinations included plasma leptin, lipoprotein profile, fasting insulin and cortisol. *Results:* Plasma leptin values were significantly increased in morbid obese patients (54.95±1.8 ng/ml) compared to those moderately obese (30.2±1.7 ng/ml; $p<0.001$) and to controls (9.77±1.4 ng/ml; $p<0.001$). Fat and age-adjusted leptin values were not different between groups. When subjects with a BMI <40 kg/m^2 were considered, plasma leptin was significantly and positively related to anthropometric variables (BMI, percentage body fat and WHR), total cholesterol, LDL-cholesterol, plasma triglycerides, AST, ALT and uric acid; and negatively with HDL-cholesterol. In contrast, when morbidly obese patients were analyzed separately, no relationships were observed between leptin concentrations and BMI, percentage of adiposity or biochemical variables. For obese patients no significant differences were observed in the adjusted leptin values with respect to the presence of diabetes, dyslipidemia or hypertension. *Conclusions:* In morbidly obese women, the plasma leptin concentrations, although increased, do not reflect the amount of adipose stores, and as such, factors other than simply adiposity need to be invoked to explain the variation in leptin values.
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INTRODUCTION

Leptin, the obesity (ob) gene product, has been implicated in body-weight regulation in mice and its deficiency associated with the development of obesity (1). In obese humans, however, increases in adipose tissue expression of leptin mRNA and, concomitantly, of circulating leptin concentrations have been demonstrated, as well as strong correlations between leptin values and various measures of obesity such as body fat or body mass index (BMI) (2-4). Rather than being seen as merely a marker of adiposity, a more active role in body-weight control has been proposed for leptin and this is inferred from the observation that different factors, physiological and/or pathological, can affect its concentrations (5-8). Most studies, even when conducted on relatively large cohorts, have been performed using lean or mildly obese subjects and, often, the higher ranges of adiposity have not been addressed. Morbid obesity represents an extreme state of the disease that only some patients attain and is estimated to occur in no more than 0.5% of obese persons, making it a relatively uncommon disorder (9). These patients, or a sub-population of them, could have some specific characteristics that predispose to

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Key words: Leptin, morbid obesity, fat mass, lipid profile, insulin.

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a progression toward the morbidly obese state. Although decreased leptin production has been reported recently in 2 severely obese children (10) and in 3 massively obese members of a Turkish family (11), the role of leptin in the development, or maintenance, of morbid obesity has not been fully investigated.

Leptin concentrations have been related to some of the metabolic risk factors associated with obesity (12-14). These relationships, however, could be artefactual with adiposity acting as a confounding factor. Hence we investigated the determinants of leptinemia in a group of morbidly obese females and compared the findings with normal-weight and mild-to-moderately obese females all of whom were weight-stable and characterized on the basis of body composition and metabolic phenotype. A secondary aim was to evaluate the relationships between leptin concentrations and other biochemical markers of metabolic derangement associated with obesity, particularly in relation to the presence of morbid obesity.

MATERIALS AND METHODS

Thirty-four morbidly obese BMI ≥40 kg/m^2 and 44 moderately obese (BMI 25-40 kg/m^2) females were consecutively recruited from among those attending the Clinics of Obesity and Nutrition at the Hospital de St. Joan de Reus. Twelve healthy, normal-weight females (BMI 19-25 kg/m^2) recruited among the medical staff served as a control group. All subjects were free of inflammatory or infectious diseases at the time of admission, had thyroid hormones and basal cortisol levels within our reference range and none were receiving anti-inflammatory medication, insulin, steroid preparations or hormone replacements. Patients were requested to maintain their normal eating habits in the week prior to admission and all of them reported being weight-stable over the previous 3 months. Obese patients were subsequently classified on the basis of metabolic complications of obesity. Type 2 diabetes was concluded when a previous diagnosis had been made or when a fasting glucose ≥7.0 mmol/l was detected. HbA_{1c} ≥7.0% was considered as diabetes under poor metabolic control. A previous or current finding of plasma cholesterol ≥6.4 mmol/l, plasma triglycerides ≥2.3 mmol/l or LDL-cholesterol (LDLc) ≥4.1 mmol/l served to establish the classification of dyslipidemia. Patients with a previous history of hypertension or 3 consecutive findings of systolic pressure ≥150 mmHg or diastolic pressure ≥95 mmHg were classified as hypertensive. Hyperuricemic patients were classified on the basis of the presence of uric acid concentrations ≥357 μmol/l or a previous diagnosis. Data on cholelithiasis were obtained from previous image analysis or from the report by the patient herself.

After admission to the metabolic ward, fasting blood samples were taken at 08:00 h for biochemical and hormone analyses. Body composition measurements were then performed. Height and weight were measured and BMI was calculated. Waist girth was measured at the minimum circumference between the iliac crest and the rib cage, hip girth at the maximum width over the greater trochanters and the waist-to-hip ratio (WHR) was then calculated. According to the consensus document of the Spanish Society for the Study of Obesity (SEEDO), abdominal fat distribution for a female population was defined as a WHR >0.9 (15). Whole body impedance at 50 kHz was measured using a tetrapolar bioelectrical impedanciometer (Human-Im Scan®, Dietosystem, Spain) early in the morning under fasting conditions and after voiding as we have described elsewhere (16). From these measurements, fat-free-mass (FFM) was calculated using the gender-specific equations validated by Segal *et al* (17). Fat mass (FM) was calculated as the difference between body weight and FFM. The mean coefficient of variation for within-patient impedance measurements in our laboratory was 0.71%. On all occasions, the observed impedance deviated from the expected value by <3.5 Ohm.

Radioimmunoassay, using commercial kits, was used to determine serum leptin concentrations (Linco Research, St. Louis, MO, U.S.A.), fasting insulin (Amersham, Little Chalfont, U.K.), and cortisol (DPC, LA, U.S.A.). Within and between assay variation were 4.98% and 4.5% for leptin, 5.05% and 13.1% for insulin, 4.31% and 5.2% for cortisol, respectively. The hospital's routine chemistry laboratory (ILab 900, Instrumentarium Laboratories, Milan, Italy) assayed plasma triglycerides and cholesterol by an enzimatic method (CHOD-POD); HDL-, LDL- and VLDL-cholesterol (HDLc, LDLc and VLDLc, respectively), fasting glucose and uric acid concentrations by a colorimetric method (ITC Diagnostics, Izasa SA); and liver function tests by a kinetic method (ITC Diagnostics, Izasa SA). HbA_{1c} was determined by a microparticle immunoassay (Abbot, Illinois, U.S.A.). To control the existence of a negative short-term energy balance, possible energy restriction was indi-

rectly evaluated by determining plasma hydroxybutyrate concentrations using an espectophotometric method (30UV kit, Sigma, St. Louis, MO, U.S.A.).

The study protocol was approved by the Ethics Committee of the Hospital de St. Joan and each subject gave written, fully-informed consent to participate. Statistical analysis was performed using the SPSS/PC package. Leptin concentrations being skewed, the values were log-transformed so as to approach a normal distribution as previously described (18, 19) and geometric means are presented here. The same procedure was used for insulin and triglyceride levels analysis. Differences in mean values between groups were assessed by one-way analysis of variance (ANOVA) and Scheffe *post-hoc* tests, and the contingency table chi-square test was used to analyze qualitative traits. Regression analyses were conducted for quantitative variables and regression coefficients (r) were derived. A multiple stepwise regression analysis was conducted to identify the factors affecting leptin concentrations and at which age, BMI, percentage body fat, WHR and insulin were entered as independent variables. The unexplained residual of leptin values in each subject was calculated by the general linear model procedure introducing age and percent body fat as covariates since these were the only variables that significantly correlated with leptin concentrations. The adjusted leptin values for each individual were calculated by adding the individual's residual leptin value to the mean leptin value of the whole group or to the mean leptin value of the obese patients when this group was analyzed separately. Statistical significance was accepted at $p<0.05$.

RESULTS

Table 1 shows the characteristics of the study group. Significant differences in all anthropometric measurements were observed between groups. Control

Table 1 - *Characteristics of the study subjects.*

	Controls (n=12)	Moderate obese (n=44)	Morbid obese (n=34)
Age (yr)	30.67 (9.5)	45.36 (11.8)*	42.0 (9.9)*
Body mass index (kg/m^2)	21.09 (1.6)	34.10 (4.0)#§	46.38 (4.3)#
Body fat (%)	26.61 (2.9)	43.60 (3.6)#§	50.71 (2.2)#
Waist-to-hip ratio	0.79 (0.04)	0.91 (0.1)#	0.88 (0.1)*
Fasting glucose (mmol/l)	4.83 (0.3)	6.12 (1.2)	6.86 (2.7)*
Plasma cholesterol (mmol/l)	4.86 (0.7)	5.77 (1.1)*	5.35 (0.8)
LDL-cholesterol (mmol/l)	2.61 (0.6)	3.62 (1.03)*	3.24 (0.6)
VLDL-cholesterol (mmol/l)	0.35 (0.2)	0.66 (0.3)*	0.63 (0.3)*
HDL-cholesterol (mmol/l)	1.91 (0.3)	1.36 (0.4)#	1.38 (0.3)#
Triglycerides (mmol/l)[a]	0.69 (0.5)	1.34 (0.6)*	1.28 (0.6)*
Urates (µmol/l)	153.4 (32.9)	248.6 (79.0)#	239.5 (63.0)#
ALT (µkat/l)	0.22 (0.05)	0.49 (0.2)*	0.48 (0.3)*
AST (µkat/l)	0.17 (0.06)	0.32 (0.2)	0.36 (0.3)*
Fasting insulin (nmol/l)[a]	0.24 (0.46)	0.48 (0.53)*‡	0.90 (0.48)#
Cortisol (nmol/l)	570.7 (294.2)	403.6 (209.2)*	349.1 (152.5)*
Leptin (ng/ml)[a]	9.77 (1.4)	30.20 (1.7)#§	54.95 (1.8)#

Results are expressed as mean (standard deviation). [a]Leptin, insulin and triglyceride values are presented as the geometric mean. *$p<0.05$ and #$p<0.001$ *vs* control subjects; ‡$p<0.005$ and §$p<0.001$ *vs* morbid obese patients (Scheffe test after significant analysis of variance).

Table 2 - *Correlation of fasting plasma leptin with body composition parameters and with hormonal or biochemical markers of metabolic derangements associated with obesity in subjects with body mass index (BMI) below or above 40 kg/m².*

	Overall study group (n=90)		BMI<40 kg/m² (n=56)		BMI≥40 kg/m² (n=34)	
	Leptin	Log leptin	Leptin	Log leptin	Leptin	Log leptin
BMI	0.56**	0.75**	0.70**	0.82**	-0.06	-0.01
Fat mass	0.56**	0.74**	0.69**	0.80**	0.016	-0.01
percent body fat	0.53**	0.77**	0.64**	0.77**	-0.06	0.03
Waist circumference	0.47**	0.72**	0.65**	0.77**	-0.28	-0.20
Waist-to-hip ratio	-0.03	0.17	0.36**	0.46**	-0.39*	-0.39*
Log fasting insulin	0.39**	0.47**	0.35**	0.35**	0.14	0.13
Plasma cholesterol	0.11	0.24*	0.37**	0.43**	-0.003	0.08
HDL-cholesterol	-0.25*	-0.41**	-0.40**	-0.51**	-0.01	-0.01
LDL-cholesterol	0.17	0.32**	0.44**	0.50**	-0.009	0.09
Log triglycerides	0.08	0.19	0.23	0.31*	-0.14	-0.20
AST	0.27*	0.35**	0.31*	0.37**	0.21	0.28
ALT	0.08	0.19	0.16	0.28*	0.01	0.06
Urates	0.27*	0.43**	0.57**	0.54**	-0.07	0.06

*$p<0.05$; **$p<0.01$.

subjects were younger than the patient groups but no differences in age were observed between the patient groups. Fasting glucose, lipid concentrations, urate and hepatic enzymes were significantly increased in patients compared to controls and slightly higher in morbid obese patients than in their moderately obese counterparts although the differences between the groups of patients were not statistically significant. Fasting insulin and plasma leptin concentrations were significantly increased in morbid obese patients compared either to control subjects or to moderately obese patients. As shown in Table 2, plasma leptin concentrations were strongly correlated with BMI, FM, percentage body fat (Fig. 1) and waist circumference but not with WHR in the overall study group. When adjusted for the degree of adiposity and age, no significant differences in leptin values between any of the groups were found.

As shown in Table 2, when subjects with BMI <40 kg/m² were considered separately, plasma leptin was strongly related to all anthropometric variables (BMI, percent body fat and WHR). Leptin was still significantly related to overall adiposity ($r=0.59$; $p<0.01$) and BMI ($r=0.67$; $p<0.01$) when the analysis was restricted to the moderately obese group. On the other hand, when the morbid obese group was analyzed separately, leptin values were inversely related only with WHR with no significant relationships with BMI or percent body fat. A positive relationship was ob-

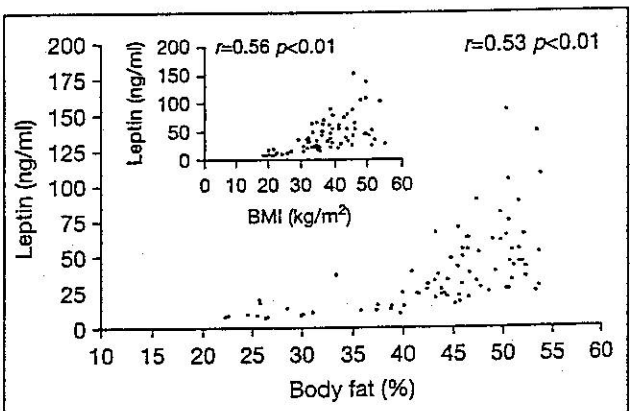

Fig. 1 - *Relationship between plasma leptin concentrations and body mass index (BMI) or percentage of body fat in the overall study group (n=90).*

served between leptin and fasting insulin for the overall study group and when subjects with BMI<40 kg/m^2 were considered, but this association was not significant when the analysis was performed separately for subjects above the BMI cut-off of 40 kg/m^2. Similarly, plasma leptin correlated significantly with lipid profiles, hepatic enzymes and uric acid concentrations in the group of subjects with BMI<40 kg/m^2 but, except for a negative correlation with fasting glucose (r=-0.39, p<0.05), no relationship between leptin and any of these biochemical variables was observed for the morbidly obese group. When adjusted for adiposity and age in the overall study group, leptin was not related to any of the biochemical nor to hormone parameters assessed and only a negative relationship with fasting glucose (r=-0.29, p<0.05) was observed.

Two morbidly obese patients admitted to some energy restriction, as did 2 from the overweight group. Two further patients in the moderately obese group, one control and one morbidly obese subject had hydroxybutyrate values beyond the ketosis range. No significant differences in absolute or adjusted leptin values were observed between these individuals and their non-ketotic counterparts and when these were excluded from the statistical analysis, the leptin values were still strongly related to adiposity in the moderately obese group but not in the morbid obesity group.

In the patient groups, 23% were classified as diabetic, 32.5% as affected from dyslipidemia, 38% as hypertensive, 9.5% as hyperuricemic, 18% had a previous history of cholelithiasis and 43% had abdominal fat distribution. Between groups, (Type 2 diabetes mellitus and hypertension were more frequent in the morbidly obese subjects than in the moderately obese (12 out of 34 *vs* 6 out of 44 and 17 out of 33 *vs* 12 out of 44 respectively; p<0.05). The frequencies of dyslipidemia and cholelithiasis were lower in the morbidly obese group than in the moderately obese group (8 out of 33 *vs* 17 out of 44 and 4 out of 31 *vs* 9 out of 42, respectively) although the differences were not statistically significant. No significant differences were observed in adjusted leptin values between diabetic patients and their non-diabetic counterparts. Among diabetic patients, only 4 were not under good metabolic control. These patients presented lower adjusted leptin levels than their well-controlled diabetic counterparts (23.44±1.19 ng/ml *vs* 41.69±1.1 ng/ml). Even after their exclusion from the statistical analysis, no differences in leptin levels were observed between diabetic and non-diabetic patients. When compared with respect to the presence or absence of abdominal fat distribution, hypertension, hyperuricemia, dyslipidemia or cholelithiasis, the adjusted leptin values were not significantly different between the patient groups (data not shown).

DISCUSSION

The results of the present study suggest that in morbidly obese females plasma leptin concentrations are not as strongly determined by total adiposity as in general populations, and, probably, other factors are more important in determining leptinemia in this population.

Several studies have described the positive relationship between leptinemia and adiposity in general populations which suggests that, in humans, serum leptin concentrations reflect the amount of body adipose tissue (3, 4, 20). A strong relationship between leptin levels and adiposity indices (BMI, percent body fat) was observed in the present study when the overall study group was assessed. However, these relationships disappear when the morbidly obese patients are assessed separately (q.v. the dispersion observed in Figure 1 of those with BMI ≥40 kg/m^2). This same lack of relationship for subjects in the high range of adiposity can be seen in the graphical data presented in published reports but with respect to which no comments appear in the text (4, 21). To-date, most of the studies have been performed in lean or mildly obese subjects and the higher ranges of adiposity have not been addressed. A recent study, conducted in a large sample of black subjects (BMI ranged 14-62 kg/m^2), reported an exponential relationship between plasma leptin and fat mass *ie* a loss of linearity in this relationship at the upper extreme of the BMI distribution curve (18). In two studies conducted on extremely obese females (12, 22) and in which, incidentally, FM had not been assessed, the reported relationship between leptin concentrations and BMI appeared weaker than expected. Further, no relationship between leptin gene expression and BMI had been observed in omental or subcutaneous adipose tissue from very obese patients (23). This suggests that metabolic responses in the morbidly obese differ considerably from those of the general population. In the present

study (in which body composition was measured) leptin concentrations appeared to be independent of the degree of adiposity for the morbidly obese group of patients. It needs be noted that the estimation of fat mass with bio-electrical impedance analysis is liable to increased inaccuracy when applied to the massively obese patient but, nevertheless, this finding is validated by the same lack of correlation observed with BMI in this group of patients. It may be suggested that this lack of correlation could be due to the narrower range of adiposity represented in the morbid obesity group. However, with respect to the moderately obese group, leptin is still strongly related to overall adiposity and BMI even though the range of BMI is narrower than that of the morbid obesity group (25.9 to 39.5 *vs* 40.3 to 55.8 kg/m^2, respectively).

Different factors are known to affect leptin concentrations. Since gender differences with respect to leptin concentrations have been well documented (24, 25), the present study was conducted only in females. In addition, short-term negative energy balance has been shown to induce acute changes in leptin values (26, 27). In the present study, energy restriction, as confirmed by hydroxybutirate determinations, did not appear to be a determinant of intergroup differences. Moreover, no clinical evidence of recent changes in body composition had been noted in our study subjects since this was a selection criterion for inclusion in the study.

Insulin has been suggested as a factor in the regulation of leptin synthesis and secretion (19, 28-30). Since the number of diabetics was significantly higher in the morbid obesity group (and with a greater degree of hyperinsulinemia) it could be that this factor induced the high variability of leptinemia despite a similar degree of adiposity in these subjects. However, the same magnitude of relationship between leptin and insulin was observed in the overweight as well as in the morbidly obese patients. Moreover, when diabetic and non-diabetic patients were compared, no differences in the adjusted leptin concentrations were observed. This finding is in agreement with previous studies such as that conducted by Haffner *et al* (31) on a large sample of diabetic and non-diabetic Mexican-Americans. Similarly, Clement *et al* (12) in a study of 241 morbidly obese patients, failed to find differences in leptin concentrations between normoglycemic, glucose intolerant and well-controlled diabetic patients of similar BMI; only patients with poorly-controlled diabetes showed significantly lower leptinemia. Accordingly to Clement *et al*, the four diabetic patients with poor metabolic control in our study presented lower adjusted leptin levels than their well-controlled diabetic counterparts. However, their exclusion from the statistical analysis, does not entail differences in leptin levels between diabetic and non-diabetic patients. Although an effect of insulin resistance on the high variability observed at the high end of the range of adiposity cannot be excluded from these data, its exact role would require further, longitudinal studies.

In our morbid obese patients, leptin concentrations were not related to indices of general adiposity but with a negative correlation only with WHR. This negative association between leptin and abdominal fat deposit in morbid obese subjects is consistent with that reported by Lönnqvist *et al* in a sub-population of females but not in males (22). Since some metabolic diseases are related to abdominal fat content, it could be suggested that, in markedly obese females, leptin could play a protective role against some metabolic derangement associated with obesity. Lönnqvist *et al* suggested that this hypothesis could be supported by the lack of relationship between leptin and markers of cardiovascular risk in morbidly obese patients. In our morbid obesity group, no relationship was observed between circulating leptin concentrations and lipid profile or insulin values. This cannot be interpreted as a direct protective effect of leptin *per se* since leptin does not correlate with these markers in the overall study group when the effect of fat mass is controlled. Moreover, when our subjects were classified on the basis of other secondary complications of obesity, no differences in leptin levels were observed irrespective of the presence or absence of any such complications.

In conclusion, in morbidly obese patients the plasma leptin concentrations although increased do not reflect the amount of adipose stores, and, as such, factors other than simple adiposity need to be invoked to explain the variation in leptin values. Whether this is the result of a distorted leptin production in massive obesity or an adaptive mechanism that prevents further weight gain when the morbid state is reached remains to be elucidated. Longitudinal studies in wider samples are needed to explore the possible protective effect in the extreme range of adiposity as well as the associated factors affecting leptin production in morbidly obese patients.

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