ISBN:978-84-691-1902-0/DL:T-355-2008

differences in antropometrical variables were observed in relation to the presence or absence of diabetes except a higher WHR in diabetic patients relative to non-diabetic patients. Diabetic patients were treated either by diet alone (n=26) or by oral anti-diabetic agents (n=15). Only one diabetic patient required insulin at the time of the present study. Good glycemic control (Hba1c≤6.5 %) was observed in 16 patients (13 with diet and 3 with oral hypoglycemic agents) and 25 patients presented poor glycemic control (13 with diet and 12 with oral hypoglycemic agents).

Fasting glucose levels were significantly increased in patients with diabetes mellitus compared to controls and their obese counterparts. Plasma total cholesterol, HDL cholesterol, VLDL cholesterol and triglycerides were increased in all groups of patients compared to controls. Increases in plasma leptin and insulin levels were observed in morbid obese patients in relation to obese and control subjects (Table 2). No significant differences were observed between groups in relation to plasma TNFα levels.

As shown in Figure 1, all patient groups showed a significant increase in both soluble plasma TNF receptors compared to control. Soluble TNFR1 was higher in morbid obese diabetic individuals compared to obese (P<0.001) or morbid obese non-diabetic patients (P=0.003). In relation to the sTNFR2, a significant mean difference was observed between the obese and the morbid obese patients (P=0.036), obese type-2 diabetic group (P=0.006), and morbid obese type-2 diabetic group (P<0.001). Morbid obese diabetic patients showed higher sTNFR2 levels than their non diabetic counterparts, although the differences were not statistically significant (P=0.065).

After adjusting for adiposity, both soluble TNF receptors were higher in obese diabetic patients (n=41; 2.11±0.09 ng/mL for sTNFR1 and 5.66±0.26 ng/mL for sTNFR2) than non diabetic obese patients (1.85±0.06 ng/mL for sTNFR1 and 4.75±0.17 ng/mL for sTNFR2; P=0.016 and

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P=0.006, respectively). Plasma leptin levels adjusted for BMI were lower in diabetic patients than in their non diabetic counterparts (33.92±3.41ng/mL vs 41.58±2.25 ng/ml) although the diference did not quite reach statistical significance (P=0.067).

Bivariate correlation analysis showed a positive relationship between both soluble plasma TNF receptors and BMI, percentage of total body-fat, fasting glucose, leptin (Figure 2) and insulin; and a negative correlation with HDL cholesterol levels (Table 3). We also observed a significant correlation between levels of sTNFR2 and WHR (Table 3). By contrast, no significant relationship was observed between plasma TNF levels and any of the quantitative variables measured.

Finally, we performed a multiple linear regression analysis using two separate models for the entire study population and for the obese subjects and, in which, plasma sTNFR1 and sTNFR2 were introduced as dependent variables while age, percentage of body fat, BMI, WHR, fasting insulin and the presence of diabetes were the independent variables. When all study subjects were considered, both soluble plasma TNF receptor levels were significantly associated with BMI and the presence or absence of diabetes (R^2=0.20, P<0.001 for sTNFR1 and sTNFR2). In obese patients, the percentage of body fat and the presence of diabetes were the only significant predictors of the both soluble TNFα receptor levels (R^2= 0.14, P<0.001 for sTNFR1 and R^2=0.15, P<0.001 for sTNFR2).

When plasma leptin levels were added to the previous model, the significant predictors of sTNFR1 in all of study subjects were leptin levels and the presence of diabetes (R^2=0.27, P<0.001). The significant predictors of sTNFR2 were leptin levels, the presence of diabetes and body fat-mass (R^2=0.23, P>0.001).

UNIVERSITAT ROVIRA I VIRGILI
EFECTO DE LA ADIPOSIDAD SOBRE EL SISTEMA TNFα-LEPTINA
Mònica Bulló Bonet
ISBN:978-84-691-1902-0/DL:T-355-2008

DISCUSSION

Increasingly in the literature of late, there has been the suggestion that TNFα could be involved in the pathogenesis of insulin resistance associated with obesity. However, there has not been any consensus and several investigators have failed to demonstrate higher circulating levels of this cytokine, in this metabolic situation, compared to controls (14,20). In our study we did not observe any significant differences in levels of this cytokine between the obese or diabetic patients and controls. The paracrine mechanism of TNFα secretion, the highly labile nature of this protein and the methodological difficulties of its quantification could be among the factors that may explain this.

The assessment of the TNFα system activity based on the plasma TNF receptor levels appears to be more reliable and of greater interest than plasma TNFα concentration itself. These proteins are easily detectable in plasma and appear to act as a buffer system prolonging the biological effects of TNFα (18,22) and seem to reflect more accurately the degree of TNFα system activation than the concentrations of circulating TNFα (22). As with other authors, we observed higher levels of both soluble TNF receptors in obese subjects and, in our study, BMI and the percentage of body fat were the best predictors of the levels of these receptors in the overall study population as well as in the obese patients considered apart.

In contrast, this is the first study to demonstrate that the levels of these receptors are higher in diabetic than in non-diabetic patients, independent of the range of adiposity. Among the anthropometric variables, age, fasting glucose/insulin, lipoprotein profile and the presence of diabetes, only the BMI and the presence/absence of diabetes were the significant predictors of both soluble forms of TNF receptors.

Some authors demonstrated a relationship between fasting insulin or HOMA IR and sTNFR1 or sTNFR2 (13,14) in obese patients. Fernández-Real et al (23) further demonstrated a negative correlation between insulin sensitivity and sTNFR2. In our study a positive and significant relationship was observed between both soluble receptors and insulin plasma levels or HOMA IR in the overall study population and this association was maintained for the sTNFR2 alone when the patient groups were assessed apart.

The increase in the levels of these receptors could be viewed as resulting from an increase in the production and activity of the TNFα system, secondary to an excess of adiposity, in order to breakdown the expansion of fat depots. Indeed, it has been observed that TNFα can decrease adipose tissue lipoprotein lipase activity (24,25) and can also induce insulin resistance through its ability to produce serine phosphorylation of insulin receptor substrate 1, thus decreasing the tyrosine kinase activity of the insulin receptor (26). Both mechanisms are responsible for a fall in adipocyte and/or muscle tissue fuel bioavailability and of an increase in lipolysis (27).

It is interesting to note that, after adjustment for adiposity, diabetic patients have lower levels of leptin than their non-diabetic counterparts. This has been previously observed by different authors (28,29) investigating obese or morbidly obese patients and can be a reflection of a decreased insulin secretion in some of these diabetic patients. Indeed, a severe insulin deficiency is likely to be present, especially in our poorly–controlled morbid obese patients since their mean fasting glycemia is uncontrolled despite treatment. As with the Clement et al study (28), after adjustment for adiposity, our diabetic patients with higher glucose levels (>10mM) had significantly lower leptin concentrations (18.62±1.18ng/mL) than the subjects with a mean fasting glucose below 10 mM (36.30±1.08 ng/mL, $p<0.001$) with a similar fasting insulin concentration (20.40±1.36 μU/mL vs 23.98±1.15 μU/mL). This suggests that the lower leptin concentrations were associated with the lack of control of diabetes. Morever, we

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observed a significant inverse relationship between glucose and leptin levels in diabetic obese patients (r =-0.432, P=0.005). The same picture is observed when diabetic patients are segregated with respect to glycemic control based on glycated hemoglobin values. The consequences of the relative hypoleptinemia observed in diabetic obese patients are unknown. Nevertheless, we can hypothesize that, especially in morbid obese patients, leptin deficiency may contribute to the maintenance of obesity and its associated metabolic complications.

Another interesting aspect of our study is the observation that there is a significant relationship between sTNFR1 and plasma leptin levels either when the overall study population is considered or only the obese patients. This is in agreement with the findings reported by Mantzoros (30) in healthy and diabetic subjects and those of Corica et al (13) in obese subjects. Our results differ from these previous studies in that we also observed a relationship between the TNFR2 concentrations and plasma leptin levels. Further, when we included the values of leptin concentrations as an independent variable in the multiple regression analysis model, both receptors can be predicted by leptin levels and the presence of diabetes.

The relationships that we observed between these proteins are in agreement with other studies in the literature and which suggest an overlapping between leptin and TNFα (7). The rationale is that, firstly, both proteins are produced by adipose tissue in proportion to the amount of fat stores (11,31) and are capable of regulating energy intake and expenditure (32). Secondly, a decrease in adipocyte expression and plasma concentrations of both proteins have been observed after weight loss (31) and these levels can increase during re-feeding/re-nutrition (11) and which indicates that similar mechanisms are involved in the regulation of these proteins. Finally, leptin synthesis can be modulated by the administration of TNFα in vitro (28,33,34) and in vivo (35), although contradictory data have been published in the literature. An increase in the adipocyte production of leptin after TNF incubation was observed by Kirchgessner et al (33). However, Zumbach et al (35) found only a transient increase in

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serum leptin production with administration of TNFα and, recently, an inhibition in leptin synthesis was reported after long-term TNF adipocyte incubation (34,36).

To conclude, TNF receptors are increased in obese and diabetic patients as a reflection of the degree of adiposity and associated insulin resistance. The independent association between plasma TNF receptors and leptin levels in obese patients is consistent with the hypothesis that these proteins are involved in the pathophysiology of obesity. Further studies are necessary to elucidate the implications of the TNFα-leptin system in the etiology and metabolic derangements associated with obesity.

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Table 1. Anthropometric and biometric characteristics of the study subjects.

	Control (n=24)	Obese (n=63)	Obese type2-DM (n=19)	Morbid Obese (n=29)	Morbid obese type2-DM (n=22)
Age (years)	38.4 (2.3)	46.3 (1.3)*	51.6 (2.6)$^{\acute{Y}}$	41.5 (2.2)$^{//}$	46.3 (1.8)*
Height (cm)	161.8 (1.3)	157.8 (0.8)*	159.0 (1.2)	158.0 (1.2)*	158.3 (1.2)
BMI (kg/m^2)	23.0 (0.5)	34.2 (0.5)$^{\acute{Y}}$	34.6 (0.7)$^{\acute{Y}}$	45.6 (0.7)$^{\acute{Y}}$,**	47.3 (1.3)$^{\acute{Y}}$,**
Body fat (%)	26.2 (0.6)	44.6 (0.3)$^{\acute{Y}}$	44.9 (0.5)$^{\acute{Y}}$	50.1 (0.3)$^{\acute{Y}}$,**	50.1 (0.4)$^{\acute{Y}}$,**
Waist (cm)	78.2 (7.8)	103.4 (11.06)$^{\acute{Y}}$	109.8(9.83)$^{\acute{Y},\acute{y}}$	119.1(11.10)$^{\acute{Y}}$,**	122.4(8.89)$^{\acute{Y}}$,**
WHR	0.81 (0.01)	0.90 (0.01)$^{\acute{Y}}$	0.94 (0.01)$^{\acute{Y},\acute{y}}$	0.85 (0.01)$^{//}$	0.91 (0.01)$^{\acute{Y},\acute{y}}$

Values are expressed as mean (SEM). Comparisons were performed using one way analysis of variance (ANOVA) with multiple comparisons. * P<0.05, $^{\acute{Y}}$P<0.001 vs control; $^{\acute{y}}$P<0.05 vs corresponding non-diabetic group; $^{//}$P<0.05, **P<0.001 vs obese or obese type2-DM.

Table 2. Biochemical characteristics of the study subjects.

	Control (n=24)	Obese (n=63)	Obese type2-DM (n=19)	Morbid obese (n=29)	Morbid obese type2-DM (n=22)
Fasting glucose (mmol/L)	5.1 (0.08)	5.6 (0.06)$^{\acute{Y}}$	8.5 (0.53)$^{\acute{Y},\S}$	5.5 (0.11)*	8.5 (0.65)$^{\acute{Y},\S}$
Plasma cholesterol(mmol/L)	5.2 (0.21)	5.9 (0.13)*	5.7 (0.24)	5.5 (0.20)	5.5 (0.20)
HDLcholesterol (mmol/L)	1.9 (0.08)	1.5 (0.05)$^{\acute{Y}}$	1.4 (0.10)$^{\acute{Y}}$	1.5 (0.07) *	1.4 (0.08)$^{\acute{Y}}$
VLDLcholesterol (mmol/L)	0.4 (0.04)	0.6 (0.03)$^{\acute{Y}}$	0.8 (0.11)*	0.6 (0.05)*	0.8 (0.09)$^{\acute{Y}}$
LDLcholesterol (mmol/L)	3.0 (0.18)	3.7 (1.13)*	3.4 (0.25)	3.3 (0.14)	3.1 (0.15)**
Triglycerides (mmol/L)	0.9 (0.08)	1.4 (0.08)$^{\acute{Y}}$	1.9 (0.27)*	1.4 (0.12)*	1.9 (0.20)$^{\acute{Y},\acute{y}}$
Fasting insulin (μU/mL)	7.58 (1.00)	14.45 (1.00)$^{\acute{Y}}$	19.05 (1.02)	28.8 (1.01)*,**	28.18(1.01)$^{\acute{Y}}$
HOMA IR	1.9 (0.24)	4.7 (0.5)$^{\acute{Y}}$	13.7 (6.6)	12.1 (2.3)*,**	7.31 (1.6)$^{\acute{Y}}$
Plasma leptin (ng/mL)	9.1 (1.00)	25.7 (1.00)$^{\acute{Y}}$	22.4 (1.01)$^{\acute{Y}}$	51.3 (1.00)$^{\acute{Y},\acute{Y}\acute{Y}}$	43.6(1.01)$^{\acute{Y}}$,**
TNFα (pg/mL)	5.7 (2.02)	11.6 (2.40)	15.3 (4.00)	8.6 (2.30)	12.0 (3.20)

Values are expressed as mean (SEM). Comparisons were performed using one way analysis of variance (ANOVA) with multiple comparisons. *P<0.05, $^{\acute{Y}}$P<0.001 vs control; $^{\acute{y}}$p<0.05, §P<0.001 vs correspondent non diabetic group; **P<0.05, $^{\acute{Y}\acute{Y}}$P<0.001 vs obese or obese type2-DM.

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Table 3. Bivariate regression analysis between plasma leptin levels, TNFα and soluble TNFα receptors and selected variables in the whole population.

	Leptin (ng/mL)	**sTNFR1 (ng/mL)**	**sTNFR2 (ng/mL)**
Age (years)	Ns	Ns	Ns
BMI (kg/cm^2)	0.738^{Ý}	0.416^{Ý}	0.356^{Ý}
WHR	Ns	Ns	0.187*
Total body fat (%)	0.724^{Ý}	0.406^{Ý}	0.356^{Ý}
Fasting glucose (mmol/L)	Ns	0.204*	0.301^{Ý}
Plasma cholesterol (mmol/L)	Ns	Ns	Ns
HDL cholesterol (mmol/L)	-0.310^{Ý}	-0.290^{Ý}	-0.328^{Ý}
VLDL cholesterol (mmol/L)	0.187*	Ns	Ns
LDL cholesterol (mmol/L)	Ns	Ns	Ns
Triglycerides (mmol/L)	0.178*	Ns	0.263^{Ý}
Leptin (ng/mL)	—	0.492^{Ý}	0.372^{Ý}
Insulin (μU/mL)	0.544^{Ý}	0276^{Ý}	0.296^{Ý}
HOMA IR	0.214^{Ý}	0.209*	0.392^{Ý}

*P<0.05; ^{Ý}P<0.001

UNIVERSITAT ROVIRA I VIRGILI
EFECTO DE LA ADIPOSIDAD SOBRE EL SISTEMA TNFα-LEPTINA
Mònica Bulló Bonet
ISBN:978-84-691-1902-0/DL:T-355-2008

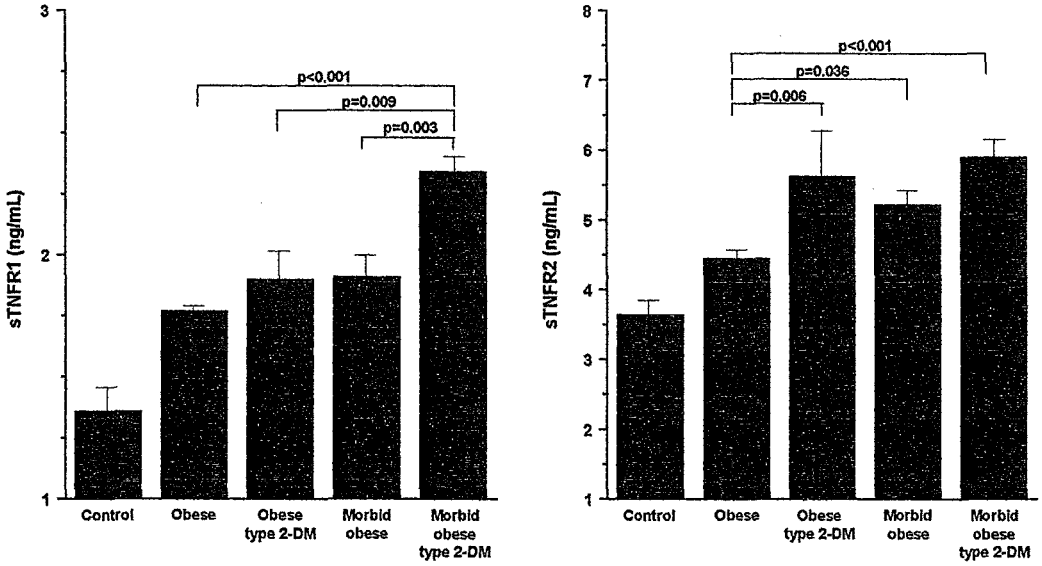

Figure 1. Plasma levels of both soluble TNFR in each study group. Mean values are significantly increased in all groups compared to controls.

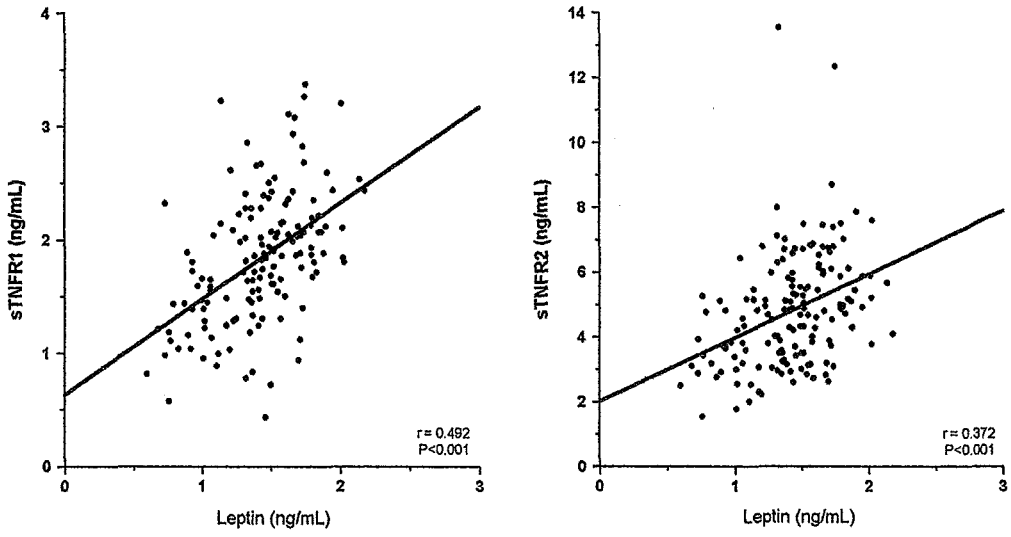

Figure 2. Relationship between the soluble TNFR and plasma leptin levels in the overall study group.

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UNIVERSITAT ROVIRA I VIRGILI
EFECTO DE LA ADIPOSIDAD SOBRE EL SISTEMA TNFα-LEPTINA
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SECCIÓN 4 .

TNF expression of subcutaneous adipose tissue in obese and morbid obese females: relationship to adipocyte LPL activity and leptin synthesis

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TNF expression of subcutaneous adipose tissue in obese and morbid obese females: relationship to adipocyte LPL activity and leptin synthesis.

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Abbreviated Title: TNF expression, leptin production and LPL activity in obese and morbid obese patients.

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UNIVERSITAT ROVIRA I VIRGILI
EFECTO DE LA ADIPOSIDAD SOBRE EL SISTEMA TNFα-LEPTINA
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Abstract

Tumor necrosis factor (TNFα) is involved in the regulation of body adiposity and insulin resistance. It has been suggested that a failure in the adipose expression of this cytokine can favour the progression of obesity in morbid obese patients. To assess whether this is the case, we measured adipocyte TNFα expression in 96 females with a wide range of adiposity and with or without type 2 diabetes. Furthermore, we analysed the relationship between TNFα expression, adipocyte LPL activity, insulin resistance and leptin in this population.

In adipose tissue, both obese and morbid obese patients presented a significant increase in TNFα and leptin expression compared to controls. Obese and morbid obese patients showed higher levels of LPL activity althought differences were not significant. We observed a significant relationship between adipose TNFα expression and body mass index ($r=0.35$ $p<0.001$). TNFα expression was negatively related to LPL activity ($r=-0.28$, $p<0.05$) and positively related to leptin expression ($r=0.35$, $p<0.001$).

Our results indicate that obese women, even those with morbid obesity, overexpress TNFα in subcutaneous adipose tissue in proportion to the magnitude of the fat depot and independently of the presence of type 2 diabetes. TNFα system may be a homeostatic mechanism that prevents further fat deposition by regulating LPL activity and leptin production.

UNIVERSITAT ROVIRA I VIRGILI
EFECTO DE LA ADIPOSIDAD SOBRE EL SISTEMA TNFα-LEPTINA
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INTRODUCTION

Obesity is a chronic condition of complex aetiology which is highly prevalent in affluent Western societies. In recent years, it has been suggested that TNFα is an important regulating factor of this condition (1,2).

Evidence provided by animal models suggests that TNFα acts as an adipostat molecule either by decreasing energy intake or by inducing thermogenesis (3,4,5). Some of the peripheral effects of TNFα are the inhibition of LPL activity (6,7,8), the down-regulating of the expression of the glucose transporter GLUT 4 (9), the inhibition of the insulin receptor activity (10,11) and the induction of adipose leptin production (12). All these effects have been described in humans, culture cells and animals. Therefore, either by direct action on LPL activity or by favouring a state of insulin resistance, TNFα produced by adipocyte may be a mechanism that helps to limit obesity.

In obese humans, it has been shown that overexpression of TNFα in adipose tissue is proportional to the extent of the fat depot, which supports the notion that TNFα is an adipostat (13,14,15). However, Kern et al. observed decreased TNFα expression in extremely obese non-diabetic subjects, suggesting that this non-production of TNFα in adipocyte is involved in the maintenance and progression of obesity in these subjects (13).

As far as we know, no other data in the literature support this intriguing finding. So, the main aim of this study was to evaluate the levels of TNFα expression in the adipose tissue of a large sample of women with considerable differences in their degree of adiposity. Some of them were morbid obese or had type 2 diabetes. We also analysed the relationship between this cytokine and adipocyte LPL activity, insulin resistance and leptin in this population.

UNIVERSITAT ROVIRA I VIRGILI
EFECTO DE LA ADIPOSIDAD SOBRE EL SISTEMA TNFα-LEPTINA
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METHODS

Subjects and sample acquisition

A group of 96 females between 18 and 65 years of age and with a wide range of adiposity (Body Mass Index (BMI) 20-64 kg/m^2) were recruited in the University Hospital of Sant Joan, Reus, Spain. The subjects were defined as obese when their body mass index was between 27 and 40 kg/m^2 (n=54), and as morbid obese when BMI≥40 kg/m^2 (n=27), according to the Spanish Consensus for obesity diagnosis (16). Patients were classified as having type 2 diabetes when a previous diagnosis had been performed or when fasting plasma glucose was >7 mmol/L on two consecutive occasions (17). The control group consisted of healthy females with a BMI between 20 and 27 kg/m^2 (n=15).

Because it was not possible to obtain enough adipose tissue sample from all the females, we priorized the measurements of leptin and TNFα expression. These measurements were taken in 83 and 78 subjects, respectively. Adipose tissue LPL activity measurements were only available in 72 subjects.

Prior to the study, subjects were put on an isocaloric diet for two days. On the day of the study, blood and adipose tissue samples were obtained after an overnight fast. Fasting blood samples were collected between 8:00 and 10:00 am. Plasma was separated immediately by centrifugation and aliquots were frozen at –80ºC for subsequent analysis. Adipose tissue samples were obtained from subcutaneous abdominal depots during elective surgical procedures such as bariatric surgery, abdominal hernia or cholecistectomy. In 36 patients, adipose tissue samples were obtained by an incisional biopsy performed in the abdominal wall. Samples of adipose tissue were quickly minced, frozen in liquid nitrogen and stored at –80ºC for further analysis.

ISBN:978-84-691-1902-0/DL:T-355-2008

All subjects were free of inflammatory or infectious diseases and none were receiving anti-obesity or anti-inflammatory drugs at the time of the study. Patients were excluded if they had neoplastic or active systemic diseases, hypothyroidism or endocrine diseases other than diabetes, or if they had been on a restrictive diet during the week before the study. The study protocol was accepted by the hospital ethical committee and subjects gave written informed consent to participate.

Body composition analysis

Weight and height were determined in all subjects, and the BMI was then calculated. Waist circumference was measured midway between the lower rib margin and the iliac crest. Hip circumference was determined as the widest circumference measured on the great trochanter. The waist-to-hip ratio (WHR) was then calculated. Whole body impedance at 50KHz was measured as previously described (18) using a tetrapolar bioelectrical impedanciometer (Human-Im-Scan Dietosystem, Spain). Fat free mass (FFM) was estimated using the gender specific equations validated by Segal (19). Fat mass was estimated by the difference between body weight and FFM.

Resting Energy Expenditure (REE) measurements

Energy expenditure measurements were determined in fasting conditions by 30-minute open-circuit indirect calorimetry (Deltatrac^{R}, Datex Instrumentation, Helsinki, Finland) as has been previously described (20). The within-individual coefficient of variation for day-to-day replicate measurements of REE was 2.3%. The observed resting energy expenditure (REEo) was calculated using Weir's equation (21). REE was adjusted by fat free mass (FFM) using the general linear model procedure. The results are expressed as kJ/day. The fasting respiratory quotient was calculated as VCO_2/VO_2.

ISBN:978-84-691-1902-0/DL:T-355-2008

Plasma biochemical analysis

Fasting plasma glucose and lipid profile (tryglicerides, total cholesterol, and high- low- and very low-density lipoprotein cholesterol) were measured enzymatically by the hospital's routine chemistry laboratory. Fasting plasma insulin was determined by a commercial radioimmunoassay kit (Amersham, Little Chalfont, UK). The intra- and inter-assay coefficients of variation were 5.05% and 13.4%, respectively and the lowest limit of detection was 48 pg/ml. The homeostasis model assessment of insulin resistance (HOMA IR) was calculated as previously described (22).

Plasma TNFα concentrations were measured by enzyme-linked immunoabsorbent assay (Pharmingen, San Diego, USA). The intra- and inter-assay coefficients of variation were <5.8% and <13.8%, respectively. Plasma concentrations of soluble TNFα receptors (sTNFR1 and sTNFR2) were determined by immonoenzymometric assay (BioSource, Fleunes, Belgium). The minimum detectable concentration was 50 pg/mL and 0.1 ng/mL, respectively. Intra- and inter-assay coefficients of variation were <6.5% and <8.9% for sTNFR1 and <3.3 % and <6.9% for sTNFR2.

Plasma leptin levels were measured by a radioimmunoenzymometric assay using a commercial kit (Linco Research, St Louis, MO). Within- and between assay variations were 4.98% and 4.5%, respectively.

RNA extraction and measurements of TNFα and leptin mRNA levels

Total RNA from 150-250 mg of subcutaneous adipose tissue was extracted with a TriPure Isolation Reagent (Boheringer Manheim GmbH, Ottweiler, Germany) using the method developed by Chomczynski and Sacchi (23). RNA quality was verified by electrophoresis in a 1.5 % agarose gel containing ethidium bromide and the RNA concentration was quantified by spectophotometry. First strand cDNA was synthesised from 0.5 μg of total RNA using random hexamers, in the presence of ditiothreitol and RNAse inhibitors. Specific primers of leptin (24)

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(GenBank D63710), TNFα (25) (GenBank X02910) and human beta-actin (26) (GenBank M10277) were derived from the gene sequences published in GeneBank. PCR reactions were performed in a thermocycler (Progene Techne, Cambridge). In a tube reaction we amplified TNFα and beta-actin by 33 cycles at 65ºC, and in another reaction we amplified leptin and beta-actin by 30 cycles at 45ºC. The resulting ethidium bromide-stained gel was imaged using the Kodac Digital Science software (Kodac, Science Park, New Haven CT). To exclude possible variations in mRNA-yield among samples, TNFα and leptin mRNA levels were expressed as specific mRNA divided by beta-actin mRNA.

Adipose tissue LPL activity

LPL activity was measured in an extract of adipose tissue as previously described by Ramirez et al (27). This activity was standarized to units of DNA, and DNA levels were determined in the same tissue homogenates by fluorimetric assay using Hoescht 33342 (IMATRA SA, Barcelona, Spain).

Statistical analysis

Statistical analysis was performed using the SPSS/PC package. Descriptive results are expressed as means and standard error (SE). Differences in mean values between groups were assessed by one-way analysis of variance. Post-hoc comparisions were carried out with the LSD test. The contingency table chi-square test was used to analyze qualitative traits. The relationship between variables was analysed by the Pearson correlation coefficient test. A step-wise multiple regression analysis was performed to identify the independent predictors of TNFα expression, with BMI, % body fat, fasting insulin, HOMA IR and the presence of diabetes as independent variables. In another model, LPL activity was introduced as the dependent variable and BMI, % body fat, TNFα expression and the presence of diabetes were introduced as independent variables. Significance was set at $p<0.05$.

ISBN:978-84-691-1902-0/DL:T-355-2008

RESULTS

The biometric characteristics of subjects are described in table 1. There were no differences in age and height between groups. In the biochemical analysis (Table 2), there were no significant differences in fasting glucose levels, plasma total cholesterol, HDL cholesterol and LDL cholesterol among the groups. Plasma VLDL cholesterol and triglycerides were increased in obese and morbid obese patients. There were no significant differences in fasting RQ and adjusted REE between groups (Table 1).

Twenty-four patients were found to be type 2 diabetics, sixteen of whom were in the obese group (29.6%) and 8 in the morbid obese group (29.6%). There were no significant differences in age, anthropometric characteristics, plasma total cholesterol, HDL cholesterol or LDL cholesterol in obese patients in relation to the presence of type 2 diabetes. Fasting insulin and HOMA IR were higher in obese and morbid obese patients (table 3). Morbid obese had a higher degree of insulin resistance than obese patients. Leptin levels and both sTNFR forms were significantly higher in obese patients than in controls. They were also higher in morbid patients than obese patients (Table 2). In contrast, there were no differences in plasma TNFα levels between groups (data not shown). When patients were grouped in relation to the presence or absence of type 2 diabetes, both sTNFR1 and sTNFR2 plasma concentrations were significantly higher in the diabetic group (2.0±0.12 ng/ml, 4.90±0.3 ng/ml, respectively) than the non-diabetic group (1.78±0.07 ng/ml and 4.31±0.18 pg/ml, respectively; $p<0.001$). Plasma leptin levels were related to both sTNFR1 and sTNFR2 ($r=0.38$, $p<0.001$ in both cases).

In adipose tissue, both obese and morbid obese patients presented a significantly higher TNFα and leptin expression than controls (Figure 1). Morbid obese patients had a higher TNFα adipose tissue expression than obese subjects, although the difference was not significant. Levels of LPL activity were higher in both obese groups (20.28±2.5 μU/ngDNA in obese group and 25.62±3.3 μU/ngDNA in morbid obese group) than in controls (14.50±3.1 μU/ngDNA) but these differences didn't reach the statistical significance. TNFα expression was higher in the

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group in which type-2 diabetes was present (Figure 1). There were no significant differences in LPL activity between the diabetic and the non-diabetic groups (20.9±2.4 μU/ngDNA in non-diabetic patients versus 25.1±3.8 μU/ng DNA in diabetic patients).

Adipose tissue TNFα expression was positively related to BMI ($r=0.35$; $p<0.005$), % body fat ($r=0.35$; $p<0.005$), leptin expression ($r=0.367$; $p<0.005$), Stnfr1 ($R=0.33$;$P<0.005$) and sTNFR2 ($R=0.30$;$P<0.01$). It was negatively related to LPL adipose tissue activity ($r=0.28$ and $r=0.31$, $p<0.05$). Both sTNFR were also related to fasting insulin ($r=0.28$ and $r= 0.31$, $p<0.005$) and HOMA IR ($r=0.32$ and $r=0.36$, $p<0.005$).

In a multiple linear regression analysis, BMI was an independent predictor of TNFα expression, and it explained 13% of its varibility ($p=0.001$). TNFα adipose tissue expression and BMI were the only significant predictors of adipose tissue LPL activity ($r^2=0.16$; $p=0.006$).

DISCUSSION

We studied a large sample of women with a wide range of adiposity in homogeneous and carefully controlled energetic metabolic conditions. Our data indicate that the adipocytes of obese patients, including those with morbid obesity, overexpressed TNFα in proportion to the magnitude of the fat depot. In fact, BMI was the main predictor of adipocyte TNFα expression throughout the population.

Our results confirm and extend the findings of Hotamisligil et al. in a small sample of 19 obese females (14). These authors described that the adipose tissue expression of TNFα was positively related to BMI, and decreased parallel to the loss in body weight. This suggests that TNFα has an important role in the pathophysiology of obesity. Kern et al. observed a positive relationship between TNFα expression in fat tissue and indices of adiposity when severely

obese patients were excluded from the analysis (13). They also reported lower TNFα mRNA levels in very obese subjects, which suggests that patients are more likely to develop obesity because the adipose tissue restricts cytokine production. Our observation that morbid patients also overexpressed TNFα in fat cells differs from the findings of Kern et al. in 1995. The heterogeneity of Kern's study sample, which included patients of both sexes and excluded type 2 diabetic subjects may partly explain such a discrepancy. In a recent study in non-diabetic subjects, Koistinen et al. reported that the adipose TNFα expression of morbid obese males was four times greater than that of lean or obese males (15). However, this overexpression was not demonstrated in obese and morbid obese females in the same study. Our study worked with the largest sample of females to date, and showed that morbid obese females presented a higher expression of TNFα in adipose tissue, independently of the presence or absence of type 2 diabetes.

It has been suggested that adipose TNFα production is a counterregulatory mechanism that acts in various ways to prevent further body fat deposition. Firstly, some authors have suggested that TNFα is an inductor of insulin resistance in experimental models (10,11,28). However, the role of adipose tissue TNFα in human insulin resistance is controversial. Two studies have demostrated that the expression of adipose tissue TNFα is not related to whole-body insulin sensitivity, either in non-diabetic obese (15) or lean subjects (29). Furthermore, the administration of an anti-TNFα antibody failed to increase insulin sensitivity in a group of type 2 diabetic patients (30). In contrast, a positive relationship between TNFα mRNA expression in adipose tissue and the level of hyperinsulinemia has been reported in both females (14) and non-diabetic males (15). Our results support the theoretical inductive role of adipose tissue TNFα in obesity-related insulin resistance. Firstly, the adipose tissue expression of TNFα was greater in diabetic patients than in their non-diabetic counterparts, although the differences were not statistically significant. On the other hand, as we have suggested before (31), the circulating concentrations of soluble TNFα were positively related to plasma insulin levels and were significantly higher in patients with type 2 diabetes.

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One of the mechanisms by which insulin resistance can limit the increase in adiposity is by modulating of LPL activity. We failed to found significant differences in LPL activity between groups, althought both, obese and morbid obese patients, presented higher values in this parameter. Moreover, in agreement with Kern et al. (13), adipose tissue LPL activity was negatively related to adipocyte TNFα expression. In fact, in our study both the expression of this cytokine and the BMI were the only significant predictors of LPL activity. This finding supports the notion that, in accordance with in vitro evidence (6,8), this cytokine has an inhibitory effect on LPL *in vivo*. Moreover, the effect of this cytokine on this enzymatic activity seems to be independent of the action of insulin since there seems to be no relationship between LPL activity and plasma insulin or HOMA IR as a parameter reflecting insulin resistance. In addition, the levels of LPL activity were not significantly different between diabetic and non-diabetic obese patients. Other authors (32,33,34) have previously suggested that LPL activity is modulated independently of insulin, probably by TNFα. This may be due to the induction of nitric oxide synthase (35) and the fact that NF-Y and octamer-binding protein (OCT-1) cease to interact with LPL DNA (36).

TNFα may also be involved in regulating fat stores through leptin, which has been shown to play a central role in energy balance regulation. TNFα has a direct effect on leptin production in adipocyte cultured cells (37), and transient increases in serum leptin have been observed after TNFα has been administered in humans (12). However, controversial *in vitro* data argue against the stimulatory effect of TNFα on adipocyte leptin production (38,39). In our study, we observed a positive relationship between the expression of these two proteins in adipose tissue. In addition, we found a positive relationship between plasma leptin and soluble TNFR levels. Whether these relationships are due to the direct action of TNFα on leptin production or to other mechanisms cannot be answered from this study. To our knowledge, only two studies have analysed the relationship between the expression of both proteins in human fat tissue: they describe either a positive (40) or a nonexistent (15) association in very small samples of obese patients.

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In conclusion, obese women overexpress TNFα in subcutaneous adipose tissue even at the extreme range of adiposity, and this overexpression is independent of the presence of type 2 diabetes. The relationship between TNFα expression, leptin production and adipose tissue LPL activity is in agreement with the hypothesis that the TNFα-system is a homeostatic mechanism that aims to limit fat depot enlargement. Further investigations are required to confirm the role of TNFα in obesity.

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Table 1. Biometric and energetic metabolic characteristics of study groups.

	Control (n=15)	Obese (n=54)	Morbid obese (n=27)
Age (years)	44.1 (3.0)	50.1 (1.3)	46.4 (2.0)
Height (cm)	159.8 (1.5)	157.5 (0.9)	158.2 (1.0)
Body mass index (kg/m^2)	24.0 (0.6)	33.7 (0.5)**	46.9 (0.9)**‡‡
Body fat (%)	27.5 (0.6)	44.6 (0.3)**	50.5 (0.2)**‡‡
Waist (cm)	80.7 (2.8)	102.9 (1.5)**	122.7 (2.1)**‡‡
Waist-to-hip ratio	0.8 (0.02)	0.9 (0.01)*	0.9 (0.01)
REE_{FFM} (kJ/day)	6461.6 (124.4)	6710.1 (93.6)	6831.3 (163.2)
RQ	0.78 (0.01)	0.77 (0.005)	0.76 (0.01)

Values are expressed as means (SE). *P<0.05, **P<0.001 vs control; ‡‡P<0.001 vs obese group.

Table 2. Biochemical and hormonal parameters in the study groups.

	Control (n=15)	Obese (n=54)	Morbid obese (n=27)
Fasting glucose (mmol/L)	5.7 (0.4)	6.4 (0.3)	6.6 (0.5)
Fasting insulin (μU/mL)	7.5 (0.6)	18.3 (2.0)*	30.4 (4.5)**‡
HOMA IR	1.9 (0.2)	5.4 (0.7)*	9.0 (1.4)**‡
Triglycerides (mmol/L)	1.0 (0.1)	1.6 (0.1)*	1.7 (0.2)*
Plasma cholesterol (mmol/L)	5.5 (0.3)	5.9 (0.1)	5.9 (0.2)
HDLcholesterol (mmol/L)	1.9 (0.1)	1.7 (0.1)	1.6 (0.1)
VLDLcholesterol (mmol/L)	0.4 (0.1)	0.7 (0.4)*	0.8 (0.1)*
LDLcholesterol (mmol/L)	3.1 (0.2)	3.6 (0.1)	3.5 (0.2)
Plasma leptin (ng/mL)	9.4 (1.5)	24.9 (1.8)*	48.5 (4.6)**‡‡
TNFR1s (ng/mL)	1.1 (0.1)	1.7 (0.1)**	2.0 (0.1)**†
TNFR2s (pg/mL)	3.2 (0.3)	4.1 (0.2)*	5.2 (0.3)**‡‡

Values are expressed as means (SE). *p<0.05, **p<0.001 vs control; ‡p<0.05, †p=0.053, ‡‡p<0.001 vs obese group.

Table 3. Adipose tissue measurements.

	Control	Obese	Morbid obese
TNF mRNA/actin	0.57 (0.1)	0.98 (0.07)*	1.16 (0.1)*
Leptin mRNA/actin	0.50 (0.1)	0.84 (0.02)**	0.91 (0.03)**
LPL (μU/ng DNA)	14.50 (3.1)	20.28 (2.5)	25.62 (3.3)

Values are expressed as means (SE). *P<0.05, **P<0.001 vs control. TNFα expression was measured in 12 control, 42 obese and 24 morbid obese females. Leptin expression was measured in 14 control, 42 obese and 27 morbid obese females. LPL activity was performed in 10 control, 42 obese and 20 morbid obese females.

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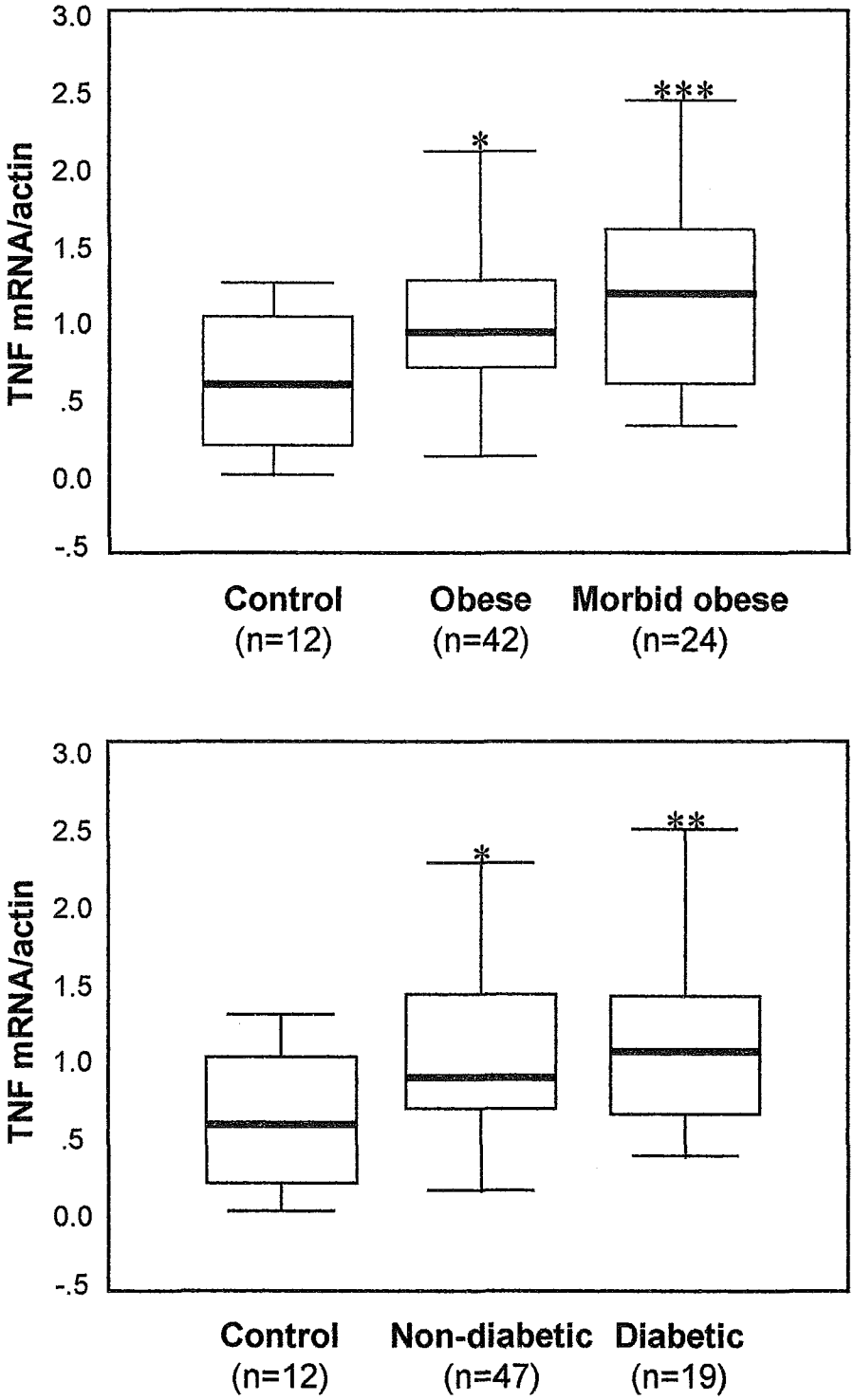

Figure 1. Levels of adipose tissue TNFα expression between groups in relation to the degree of obesity (upper panel) and the presence or absence of type 2 diabetes (lower panel). *p<0.05, **p<0.01, ***p<0.005, vs control group.

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SECCIÓN 5 .

Patrón inflamatorio en mujeres con obesidad y diabetes tipo 2. (En preparación)

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INTRODUCCIÓN

La obesidad es una condición crónica de etiología multifactorial que por su creciente prevalencia y por la importancia de las complicaciones metabólicas asociadas, puede convertirse en los próximos años, en un importante problema de salud pública.

Esta patología, caracterizada por un contenido excesivo de grasa corporal, se ha asociado recientemente a un estado inflamatorio crónico del tejido adiposo, definido sobretodo por un incremento de la expresión adipocitaria de algunas citoquinas proinflamatorias como el TNFα o la IL-6 (Hotamisligil 1995, Mohamed-Ali 1997). Estas citoquinas, y probablemente también la leptina (Wang 1997), son capaces de inducir, a nivel hepático, la síntesis de proteínas de fase aguda y provocar por tanto un cierto grado de inflamación sistémica. Estudios recientes han observado que la concentración sérica de proteína C reactiva y la velocidad de sedimentación globular, principales marcadores de la reacción de fase aguda, se encuentran sustancialmente elevados en aquellos pacientes que presentan obesidad, siendo el índice de masa corporal el principal factor predictor de la variabilidad observada para la PCR (Visser 1999, Hak 1999). El incremento de la expresión adipocitaria de TNFα o de IL-6 descrito en el paciente obeso podría explicar las elevadas concentraciones de PCR que se observan es este tipo de sujetos (Mohamed-Ali 1997, Fried 1998). De hecho, se ha descrito una asociación positiva entre la concentración de IL-6 en el tejido adiposo y las concentraciones plasmáticas de PCR en pacientes obesos (Bastard 2000). A pesar de que la leptina se ha propuesto como una sustancia capaz de modular la respuesta inflamatoria mediante la inducción de la fagocitosis y la expresión de citoquinas proinflamatorias (Lofreda 1998, Lord 1998) queda por determinar el efecto de la sobreexpresión adipocitaria de leptina sobre algunos reactantes de fase aguda. Finalmente cabe destacar también que diversas patologías como la artritis reumatoide, el síndrome de apneas del sueño, la enfermedad cardiovascular isquémica o la diabetes tipo 2, caracterizadas también por presentar unos niveles sustancialmente elevados de marcadores y

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mediadores de inflamación como la proteína C reactiva, la leptina y el TNFα, se asocian frecuentemente a la obesidad.

En este estudio pretendemos pues evaluar el comportamiento de algunos marcadores de inflamación en función del grado de adiposidad corporal y la presencia de diabetes tipo 2, así como determinar la relación de estos parámetros con los niveles plasmáticos de leptina y receptores solubles de TNFα, y con la expresión adipocitaria de leptina y TNFα.

MATERIAL Y MÉTODOS

Sujetos de estudio

En este estudio participaron un total de 146 mujeres de entre 18 y 65 años distribuidas en tres grupos en función de grado de adiposidad: grupo control (IMC entre 20 y 27 kg/m^2), grupo obesidad (IMC entre 27 y 40 kg/m^2) y grupo obesidad mórbida (IMC$\geq$40 kg/m^2) . Las pacientes obesas fueron también clasificadas en función de la presencia de diabetes tipo 2, considerándose diabéticas aquellas pacientes con diagnóstico previo o cuando los niveles de glucosa fueran $\geq$7 mmol/L en las dos últimas determinaciones consecutivas. Las características biométricas y antropométricas de las pacientes de estudio se resumen en la tabla 1.

Mediante cirugía bariátrica, corrección de eventraciones, colecistectomías y biopsias se obtuvo una muestra de tejido adiposo subcutáneo de aproximadamente 300 mg en un subgrupo de 76 mujeres.

Todas las pacientes fueron informadas de la totalidad de las pruebas a realizar y consintieron por escrito a participar en el estudio. Ninguna de estas pacientes presentaba enfermedades infecciosas o inflamatorias en el momento de estudio, excluyéndose aquellas mujeres que

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presentaron valores leucocitarios iguales o superiores a $11*10^9$/L. No se incluyeron las pacientes con enfermedades neoplásicas o sistémicas, hipotirodismo u otras alteraciones endocrinas, exceptuando la diabetes tipo 2.

Este estudio fue evaluado por el Comité de Ética del Hospital de Sant Joan de Reus el cual emitió una resolución favorable.

Métodos

A todas las pacientes se les determinó la talla con una precisión de 0,5 cm y el peso con precisión de 0,1kg. El índice de masa corporal (IMC) se determinó según el cociente peso/talla^2. El perímetro de la cintura se midió a la altura del punto medio entre la última costilla y la cresta iliaca. El diámetro de la cadera se midió alrededor de las nalgas, a la altura de la sínfisis del pubis. La masa libre de grasa se determinó mediante impedancia bioeléctrica tetrapolar a 50 Khz (Human-I Scan^R, Dietosystem, Spain) utilizando las ecuaciones de Segal específicas por sexo y grado de adiposidad (Segal 1998). La masa grasa se calculó a partir de la diferencia entre el peso corporal y la masa libre de grasa.

A todas las pacientes se les determinaron los niveles sanguíneos de glucosa, albúmina, fibrinógeno y la fórmula leucocitaria mediante técnicas de rutina hospitalaria. Las concentraciones plasmáticas de leptina (Llinco Research, St Louis, MO) e insulina (Amersham, Little Chalfont, UK) se determinaron mediante un RIA comercial. El HOMA IR se calculó según la fórmula previamente descrita por otros autores (Corica 1999)

HOMA IR = [insulina plasmática (μU/mL) * glucosa plasmática (mmol/L)] / 22.5

Las concentraciones de receptores solubles de TNFα fueron determinadas mediante técnicas inmunoenzimométricas comerciales (Pharmingen, San Diego, USA). La cuantificación de mRNA se obtuvo mediante una RT-PCR. Los niveles de mRNA de TNFα y leptina se normalizaron expresándose en función del mRNA específico para la beta-actina humana. El diámetro medio

ISBN:978-84-691-1902-0/DL:T-355-2008

adipocitario fue determinado en un total de 40 pacientes mediante técnicas de microscopía óptica y el posterior análisis morfométrico. Se consideró hipertrofia cuando el diámetro estimado era superior al percentil 50.

Análisis estadístico

Los resultados descriptivos de las variables contínuas se expresan como la media±error estándar. Las comparaciones entre grupos se realizaron mediante una ANOVA. Para los contrastes post-hoc se aplicó el test LSD. La relación entre las distintas variables se analizó mediante el coeficiente de correlación de Pearson. El ajuste de las variables se realizó mediante una ANCOVA. Para el análisis estadístico se utilizó el programa SPSS/PC. La significación estadística se consideró cuando $p<0,05$.

RESULTADOS

Las características biométricas y antropométricas de las pacientes de estudio se resumen en la tabla 1. Como se muestra en la tabla 2 los niveles de albúmina disminuyen en los grupos con obesidad cuando se comparan con el grupo control. La velocidad de sedimentación globular, las concentraciones plasmáticas de fibrinógeno, el recuento leucocitario total y el número de neutrófilos difieren significativamente entre los tres grupos de estudio, siendo significativamente más altos en los pacientes obesos, particularmente en los obesos mórbidos.

Cuando únicamente consideramos la población de mujeres obesas clasificadas en función de la presencia de diabetes tipo 2 (n=121), y tras ajustar los parámetros antropométricos por las diferencias de adiposidad, únicamente las concentraciones plasmáticas de albúmina diferían entre pacientes diabéticos y no diabéticos (tabla 3).

ISBN:978-84-691-1902-0/DL:T-355-2008

El IMC, el porcentaje de grasa y el cociente cintura-cadera se relacionaron positivamente con el número total de leucocitos y los neutrófilos, y negativamente con la albúmina plasmática. El IMC se relacionó también positivamente con el fibrinógeno en plasma, el recuento de monocitos y con la velocidad de sedimentación globular (Tabla 4). No se observaron diferencias significativas en ninguno de estos parámetros inflamatrios en función de la presencia de hipertrofia adipocitaria (datos no mostrados).

La leptina plasmática y ambas formas de receptores solubles presentaron una relación directa con el fibrinógeno y la VSG e inversa con la albúmina. Los receptores solubles de TNFα se relacionaron también significativa y positivamente con el número de leucocitos totales, con el número de neutrófilos y con la leptina (Tabla 5).

La expresión adipocitaria de TNFα y leptina se relacionó positivamente con el fibrinógeno, los leucocitos y los neutrófilos. No obstante únicamente la expresión de leptina adipocitaria presentó una asociación negativa con los niveles plasmáticos de albúmina. Los niveles de expresión de ambas proteínas se relacionaron también entre sí ($r=0,323$ $p<0,001$), y con los niveles plasmáticos de leptina , sTNFR1 y sTNFR2 (Tabla 6).

En un análisis de regresión múltiple en el cual se introdujo la albúmina como variable dependiente y el IMC, la presencia de diabetes tipo 2, y las concentraciones plasmáticas de ambos receptores solubles para el TNFα fueron introducidos como variables independientes, el IMC y el sTNFR1 permitían explicar el 20% de la variabilidad observada en los niveles de albúmina. El IMC fue también un factor determinante de la variabilidad observada en las concentraciones de fibrinógeno ($r^2=0,216$ $p<0,001$).

ISBN:978-84-691-1902-0/DL:T-355-2008

DISCUSIÓN

Diversos estudios recientes publicados en la literatura han demostrado un significativo incremento de algunos marcadores de inflamación, como el TNFα, la interleuquina 6 o la proteína C reactiva, en función del grado de adiposidad y la resistencia a la insulina o la diabetes tipo 2 (Kern 1995, Fried 1998, Yudkin 1999). Pasulka y col observaron también un incremento de la VSG en un grupo reducido de pacientes con obesidad mórbida (Pasulka). Así mismo, algunos autores han observado una disminución del número de leucocitos totales, neutrófilos, linfocitos y monocitos tras la pérdida ponderal debida a la administración de una dieta muy baja en calorías (VLCD) (Field 1991, Blanch 1993).

En nuestro estudio evaluamos el comportamiento de otros reactantes de fase aguda positivos como el fibrinógeno y la velocidad de sedimentación globular, y negativos como la albúmina, en un grupo amplio de mujeres distribuidas en función del grado de adiposidad y la presencia de diabetes tipo 2. La relación observada entre estos parámetros y el grado de obesidad, es una evidencia más de que ésta se asocia a un cierto estado de inflamación sistémica. Por otra parte observamos que la disposición predominantemente visceral de la grasa se asocia de manera más importante a este estado de inflamación sistémica. Este hallazgo confirma lo que otros autores habían sugerido previamente (Hak 1999, Visser 1999).

En estudios realizados en población general, la resistencia a la insulina se ha asociado también a un incremento de la respuesta de fase aguda (Pickup JC 1997, Yudkin JS). Contrariamente, en un estudio realizado por Hak y colaboradores la relación observada entre los niveles circulantes de proteína C reactiva y la resistencia a la insulina desaparece tras ajustar la PCR por las diferencias en el IMC, siendo el IMC el único factor predictor de la variabilidad observada en la concentración de PCR (Hak 1999). Del mismo modo, en nuestro estudio observamos que tras corregir los diversos marcadores de inflamación por las diferencias de adiposidad, únicamente la albúmina mostraba diferencias significativas en función de la

ISBN:978-84-691-1902-0/DL:T-355-2008

presencia de diabetes tipo 2. Así mismo, tras ajustar estas variables por las diferencias en la insulina circulante, se observaron diferencias significativas en el recuento de neutrófilos, leucocitos y las concentraciones de fibrinógeno entre ambos grupos de pacientes con obesidad (datos no mostrados).

Los mecanismos bioquímicos y moleculares implicados en la respuesta inflamatoria asociada a la obesidad no están todavía bien caracterizados. Sin embargo, algunos autores han sugerido que el incremento de la expresión de TNFα, habitualmente observada en el tejido adiposo del paciente obeso, podría inducir la síntesis de IL-6 (Stephens 1992). Esta sería liberada a la circulación pudiendo estimular la producción hepática de algunas proteínas de fase aguda (Heinrich 1990) Por otro lado, la identificación de receptores hepáticos para la leptina (Wang 1997), así como las relaciones positivas observadas entre la leptina y la IL-6 (Pickup 2000) o la posible existencia de un sistema TNFα-leptina (Bulló 2000), sugieren que esta proteína puede ejercer también un papel importante en la modulación de la respuesta inflamatoria asociada a la obesidad. No obstante y hasta la actualidad, ningún estudio ha evaluado el comportamiento de la expresión adipocitaria de ambas proteínas y su relación con los reactantes plasmáticos de fase aguda. En una población suficientemente amplia de mujeres con diversos grados de adiposidad observamos una relación positiva entre la expresión génica de TNFα y los niveles plasmáticos de sTNFR. Así mismo, ambos parámetros se relacionaron con los parámetros inflamatorios analizados, siendo estos resultados coherentes con las evidencias experimentales que sugieren que la sobreexpresión adipocitaria de citoquinas podría ser la responsable de desencadenar una reacción inflamatoria sistémica en los pacientes con obesidad. El hecho de que la pérdida de peso se acompañe de una disminución en la expresión adipocitaria de TNFα, incluso en los pacientes diabéticos, apoyarían también esta hipótesis (Hotamisligil 1994, Katsuki 1998). Nuestros resultados contrastan con la pérdida de relación entre la concentración de sTNFR2 y el número de leucocitos y neutrófilos descrita por Huang y colaboradores (Huang 2000). Estas discrepancias pueden ser debidas a

ISBN:978-84-691-1902-0/DL:T-355-2008

que este estudio estaba realizado en un grupo de hombres y mujeres muy heterogéneo no bien caracterizado en función del grado de adiposidad.

Por otra parte observamos que tanto la expresión de leptina como los niveles circulantes de esta proteína se relacionaban positivamente con la expresión adipocitaria de TNFα y con los niveles plasmáticos sTNFR, así como con determinados parámetros inflamatorios. Estos resultados apoyarían el posible papel de la leptina como modulador de la respuesta inflamatoria de fase aguda asociada a la obesidad.

Los resultados obtenidos en este estudio apoyan la hipótesis de que la obesidad puede asociarse a un proceso inflamatorio del tejido adiposo capaz de inducir una respuesta periférica de fase aguda, independientemente de la presencia de diabetes tipo 2. En un futuro será necesario aclarar el papel que estas alteraciones inflamatorias ligadas al acúmulo adiposo pueden ejercer sobre la aparición y mantenimiento de las complicaciones metabólicas ligadas a la obesidad.

UNIVERSITAT ROVIRA I VIRGILI
EFECTO DE LA ADIPOSIDAD SOBRE EL SISTEMA TNFα-LEPTINA
Mònica Bulló Bonet
ISBN:978-84-691-1902-0/DL:T-355-2008

Tabla 1. Característica biométricas y antropométricas de la población de estudio

	CONTROLES (n=25)	OBESIDAD (n=77)	OB MÓRBIDA (n=44)
Edad (años)	38,6 (2,6)	48,1 (1,2)**	44,4 (1,6)*
Talla (cm)	160,8 (1,4)	158,0 (0,7)	157,8 (0,9)
IMC (kg/cm^2)	23,04 (0,5)	34,4 (0,4)**	46,4 (0,7)**##
%grasa	27,7 (0,6)	44,7 (0,3)**	50,6 (0,2)**##
C/C	0,81 (0,02)	0,90 (0,01)**	0,87 (0,01)*#
Diámetro adipocitario	121,2 (6,1)	116,3 (4,0)	127,3 (9,5)

*p<0,05 **p<0,001 respecto al grupo control; #p<0,05 ##p<0,001 respecto al grupo obesidad

Tabla 2. Reactantes de fase aguda en la población de estudio

	CONTROLES (n=25)	OBESIDAD (n=77)	OB MÓRBIDA (n=44)
Albúmina (g/L)	45,5 (0,7)	42,6 (0,4) ^{H}	42,2 (0,6)**
VSG (mm/h)	8,0 (0,7)	14,5 (1,5)*	18,1 (1,5)* $^{\beta}$
Leucocitos (10^9/L)	5,7 (0,3)	6,6 (0,1) ^{H}	7,2 (0,2)**#
Limfocitos (10^9/L)	1,9 (0,1)	2,1 (0,06)	2,1 (0,07)
Monocitos (10^9/L)	0,4 (0,03)	0,5 (0,02)*	0,5 (0,01)*
Neutrófilos (10^9/L)	3,21 (0,2)	3,8 (0,1)*	4,4 (0,2)**##
Fibrinógeno (g/L)	3,5 (0,1)	4,01 (0,09)*	4,7 (0,2)**##

* p<0,05 $^{\Psi}$ p=0,053 ^{H} p<0,005 ## p<0,001 respecto al grupo control; # p<0,05 $^{\beta}$ p<0,005 ##p<0,001 respecto al grupo obesidad

Tabla 3. Reactantes de fase aguda en función de la presencia de diabetes

	No diabéticos (n=79)	Diabéticos (n=42)
Albúmina (%grasa)	43,1 (4,6)	41,2 (4,1)*
Fibrinógeno (%grasa)	4,2 (1,2)	4,3 (1,1)
VSG (mm/h)	14,8 (1,8)	15,2 (3,1)
Leucocitos (10^9/L)	6,6 (0,2)	7,2 (0,3)
Neutrófilos (10^9/L)	3,9 (0,1)	4,4 (02)

Todos los parámetros están ajustados por el porcentaje de masa grasa. *p<0,05

UNIVERSITAT ROVIRA I VIRGILI
EFECTO DE LA ADIPOSIDAD SOBRE EL SISTEMA TNFα-LEPTINA
Mònica Bulló Bonet
ISBN:978-84-691-1902-0/DL:T-355-2008

Tabla 4. Marcadores de inflamación en función del grado y la distribución de la adiposidad

	Monocitos	Neutrófilos	Fibrinógeno	Leucocitos	Albúmina	VSG
IMC (kg/cm^2)	0,173*	0,323**	0,453**	0,351**	-0,318**	0,248**
%grasa	0,140	0,269*	0,362**	0,317**	-0,383**	0,215$^{\varpi}$
C/C	0,201*	0,173 $^{\#}$	-0,104^{ns}	0,216*	-0,194*	0,044 ^{ns}

*p<0,05 $^{\varpi}$p=0,05 **p<0,001 ^{ns}=no significativo

Tabla 5. Relación entre la leptina y los niveles solubles de TNF con los marcadores de inflamación

	Albúmina	VSG	Leucocitos	Neutrófilos	Fibrinógeno	Leptina
sTNFR1	-0,399**	0,284**	0,253**	0,204*	0,244**	0,675**
sTNFR2	-0,307**	0,174**	0,168*	0,195*	0,306**	0,438**
Leptina	-0,355**	0,227**	0,133 ^{ns}	0,103 ^{ns}	0,327**	

*p<0,05 **p<0,001 ^{ns}=no significativo

Tabla 6. Relación entre la expresión adipocitaria de leptina y TNF con los marcadores de inflamación

	Albúmina	Leucocitos	Neutrófilos	Fibrinógeno	Leptina	sTNFR1	sTNFR2
mRNATNF	-0,222 ^{ns}	0,314**	0,348*	0,244*	0,141^{ns}	0,306**	0,287**
mRNALep	-0,303**	0,324**	0,274*	0,354**	0,275*	0,430**	0,373**

*p<0,05 **p<0,001 ^{ns}=no significativo

ISBN:978-84-691-1902-0/DL:T-355-2008

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UNIVERSITAT ROVIRA I VIRGILI
EFECTO DE LA ADIPOSIDAD SOBRE EL SISTEMA TNFα-LEPTINA
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DISCUSIÓN

UNIVERSITAT ROVIRA I VIRGILI
EFECTO DE LA ADIPOSIDAD SOBRE EL SISTEMA TNFα-LEPTINA
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DISCUSIÓN

En el momento de plantearse esta tesis se habían descrito en la literatura tres sustancias sintetizadas en el adipocito, la leptina, el TNFα y la oleoil-estrona, que podrían desempeñar un papel importante en el control metabólico de las reservas grasas. Por ello, nos propusimos estudiar ciertos factores posiblemente determinantes de los niveles de estas sustancias en plasma y en el adipocito en un grupo poblacional homogéneo en cuanto al sexo (consideramos únicamente mujeres), distribuido en un amplio rango de adiposidad y de sensibilidad a la insulina para responder a las siguientes preguntas:

1. ¿La adiposidad condiciona los niveles de TNFα y leptina en plasma así como la expresión de estas proteínas en el adipocito? Y si es así, ¿podemos observar diferencias entre los obesos y los obesos mórbidos?

Mientras que la concentración plasmática de TNFα es frecuentemente indetectable en el plasma de la población general e incluso en determinadas situaciones inflamatorias, los niveles circulantes de los receptores solubles del TNFα se detectan habitualmente en suero o plasma. Parte de estas diferencias son atribuibles a la mayor labilidad del TNFα pero sobretodo es debida a que su concentración es sustancialmente más elevada (Heney 1995). Puesto que por un lado la mayoría de estudios realizados al respecto atribuyen a los receptores solubles un papel estabilizador del trímero activo de TNFα (Aderka 1992), y por otro lado se ha sugerido que éstos deben ser un buen reflejo del grado de activación del TNFα (Bemelmans 1996), todos los análisis referidos a la actividad del TNFα han sido realizados mediante el estudio de

estos receptores solubles.

En nuestro estudio observamos una relación positiva y significativa entre los parámetros indicadores de adiposidad, como el índice de masa corporal o el porcentaje de grasa, y las concentraciones plasmáticas de los receptores solubles de TNFα (sTNFR1 y sTNFR2), siendo el índice de masa corporal uno de los factores que explican la variabilidad observada en ambas proteínas. Esta mayor concentración plasmática de receptores solubles de TNFα se mantenía incluso en los rangos altos de adiposidad, apreciándose diferencias significativas entre las mujeres con obesidad moderada y aquellas que presentaban obesidad mórbida (ver Sección 3).

El efecto de la adiposidad corporal sobre la expresión y síntesis de ambos receptores solubles de TNFα había sido previamente descrita por otros autores. No obstante, estos estudios se habían realizado en grupos poblacionales más reducidos y no bien caracterizados en función del grado de adiposidad (Hotamisligil 1997, Fernández-Real 1998) o bien en grupos heterogéneos considerando conjuntamente, hombres y mujeres (Corica 1999). Así mismo, los primeros estudios que evaluaban el efecto de a adiposidad sobre los receptores solubles de TNFα únicamente observaban diferencias significativas respecto al sTNFR2 (Hotamisligil 1997, Fernández-Real 1998). Por tanto nuestro estudio es, hasta el momento, el primero que ha analizado conjuntamente ambas formas de receptores solubles en una gran población de mujeres distribuidas en un amplio rango de adiposidad.

Sin embargo, puesto que no disponemos de los niveles de expresión adipocitaria para los receptores del TNFα, no podemos determinar si las concentraciones observadas en plasma son un reflejo del grado de producción en el adipocito o bien son el resultado de la síntesis en otras células, como por ejemplo los monocitos, los cuales parecen también aumentar numéricamente en la obesidad. Un estudio reciente de Hube y colaboradores ha descrito una mayor expresión adipocitaria de TNFR1 y de TNFR2 en los pacientes que presentan obesidad

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respecto a los individuos normopeso (Hube 1999). Por tanto, y a pesar de que estos autores no determinaron las concentraciones circulantes de las formas solubles de receptor, no pudiendo pues determinar la relación que se establece entre la tasa de producción adipocitaria y la liberación plasmática, parece razonable pensar que buen parte de las concentraciones plasmáticas que observamos en la obesidad sean debidas a un sobreexpresión adipocitaria de ambos receptores

La expresión adipocitaria de TNFα incrementaba también en función del tamaño de las reservas grasas, siendo la expresión de esta citoquina más elevada en los pacientes que presentan obesidad mórbida respecto al grupo control y al grupo con obesidad moderada (ver artículo 4). A pesar de que no observamos diferencias significativas entre los dos grupos de obesidad, la estrecha relación observada entre el IMC y la expresión de TNFα, demuestran que la producción adipocitaria de esta citoquina depende, en gran medida, del grado de adiposidad. Estos resultados contradicen parcialmente los observados por Kern y colaboradores. Así pues, mientras que estos autores observaron también una relación significativa entre la adiposidad y la expresión de TNFα en los pacientes con IMC<40 kg/m^2, los niveles de expresión de esta citoquina en aquellos pacientes que presentaban obesidad mórbida, eran menores de lo esperado según el IMC (Kern 1995). Por ello, sugirieron que la obesidad mórbida podría ser el reflejo del fracaso en la producción adipocitaria de esta citoquina con importantes acciones sobre el metabolismo energético. La discrepancia que existe entre nuestros resultados y los obtenidos por Kern podría ser debida a diferentes factores, como por ejemplo la heterogeneidad respecto al sexo que mostraba la población de Kern y el reducido número de pacientes estudiados por este autor (39 pacientes distribuidos en un rango de adiposidad entre 20,2-57,6 kg/m^2).

El conjunto de todos estos resultados permite afirmar que el sistema del TNFα se comporta en el hombre como un buen indicador del tamaño de las reservas grasas, incluso en aquellos sujetos con obesidad severa. Además, los resultados de nuestro estudio no apoyan la hipótesis

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del fallo en la producción de TNFα como causa de obesidad mórbida tal y como había sido sugerido por algunos autores (Kern 1995).

Desde que se identificó la leptina, algunos estudios han observado que las concentraciones plasmáticas de esta proteína en poblaciones sanas, son proporcionales a las reservas grasas del organismo (Lönnqvist 1995, Maffei 1995, Considine 1996). La relación que nosotros observamos entre el IMC o el porcentaje de grasa corporal y los niveles circulantes de leptina sustentan esta afirmación (ver Sección 1). Resultados similares fueron obtenidos también tras evaluar la expresión de leptina a nivel adipocitario. Así pues, pudimos observar una relación positiva entre la expresión de esta proteína en el adipocito y los distintos indicadores de adiposidad estudiados (ver Sección 4). Sin embargo, las pacientes que presentaban obesidad mórbida mostraron una gran dispersión en las concentraciones plasmáticas de leptina. De hecho, cuando analizamos de forma independiente el grupo de pacientes con un IMC superior a 40 kg/m^2, ninguno de los parámetros indicadores de la adiposidad se relacionó con los niveles de esta proteína en plasma. Este comportamiento se reflejó también a nivel adipocitario, puesto que no observamos diferencias significativas respecto a la expresión adipocitaria de leptina entre los dos grupos de mujeres con obesidad. Esta pérdida de relación entre la leptina plasmática y el IMC o el porcentaje de grasa en los rangos altos de adiposidad había sido observada gráficamente en dos estudios previos (Zimmet 1996, Considine 1996). Sin embargo, ninguno de estos autores había realizado ninguna observación al respecto.

Nuestros resultados muestran también una asociación negativa y significativa entre los niveles plasmáticos de leptina y el índice cintura-cadera cuando la población estudiada fue clasificada en función de la presencia o no de obesidad severa (IMC> o < a 40kg/m^2). En el grupo de mujeres con obesidad moderada observamos que los niveles de leptina se corelacionaban con el patrón lipídico plasmático (colesterol total, colesterol LDL, colesterol HDL y triglicéridos) así como con otros parámetros (γGT, GOT, ácido úrico) frecuentemente alterados en la obesidad. Sin embargo, estas relaciones desaparecían en las pacientes que presentaban obesidad