Resistin, Adiponectin, Ghrelin, Leptin, and Proinflammatory Cytokines: Relationships in Obesity

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Abstract

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Objective: To evaluate interactions among leptin, adiponectin, resistin, ghrelin, and proinflammatory cytokines [tumor necrosis factor receptors (TNFRs), interleukin-6 (IL-6)] in nonmorbid and morbid obesity.

Research Methods and Procedures: We measured these hormones by immunoenzyme or radiometric assays in 117 nonmorbid and 57 morbidly obese patients, and in a subgroup of 34 morbidly obese patients before and 6 months after gastric bypass surgery. Insulin resistance by homeostasis model assessment, lipid profile, and anthropometrical measurements were also performed in all patients.

Results: Average plasma lipids in morbidly obese patients were elevated. IL-6, leptin, adiponectin, and resistin were increased and ghrelin was decreased in morbidly obese compared with nonmorbidly obese subjects. After adjusting for age, gender, and BMI in nonmorbidly obese, adiponectin was positively associated with HDLc and gender and negatively with weight ($\beta = -0.38$, $p < 0.001$). Leptin and resistin correlated positively with soluble tumor necrosis factor receptor (sTNFR) 1 ($\beta = 0.24$, $p = 0.01$ and $\beta = 0.28$, $p = 0.007$). In the morbidly obese patients, resistin and ghrelin were positively associated with sTNFR2 ($\beta = 0.39$, $p = 0.008$ and $\beta = 0.39$, $p = 0.01$). In the surgically treated morbidly obese group, body weight decreased significantly and was best predicted by resistin concentrations before surgery ($\beta = 0.45$, $p = 0.024$). Plasma lipids, insulin resistance, leptin, sTNFR1, and IL-6 decreased and adiponectin and ghrelin increased significantly. Insulin resistance improved after weight loss and correlated with high adiponectin levels.

Discussion: TNFα receptors were involved in the regulatory endocrine system of body adiposity independently of leptin and resistin axis in nonmorbidly obese patients. Our results suggest coordinated roles of adiponectin, resistin, and ghrelin in the modulation of the obesity proinflammatory environment and that resistin levels before surgery treatment are predictive of the extent of weight loss after bypass surgery.

Key words: inflammation, insulin resistance, adipokines, sTNFRs, TNF system

Introduction

Obesity is a major risk factor for insulin resistance, type 2 diabetes, heart disease, orthopaedic problems, and many other chronic diseases. The incidence of obesity has dramatically increased and has become epidemic in the western world (1). The etiology is multifactorial, with genetic, environmental, socioeconomic, and behavioral or psychological influences, with an increase in the related morbidity and mortality (2). Obesity is the final consequence of a chronic positive energy balance, regulated by a complex network between endocrine tissues and the central nervous system (3,4).

Fat tissue is increasingly viewed as an active endocrine organ with a high metabolic activity. Adipocytes produce and secrete several proteins that act as veritable hormones,

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responsible for the regulation of energy intake and expenditure (5). Many of these hormones, collectively called adipokines, play important roles in the inflammatory and atherosclerotic processes. These include tumor necrosis factor (TNF)[1] α, leptin, interleukin (IL) 6, angiotensinogen, and plasminogen activator inhibitor-1. Increasingly evidence indicates that adiposity contributes to a proinflammatory milieu (6). Reduction in fat mass correlates with decrease in the serum levels of many of these proinflammatory adipokines, implying that approaches designed to promote fat loss should be useful in attenuating the proinflammatory environment associated with obesity (6). More recently, adiponectin and resistin have been described as secretory products of the adipose tissue (7).

Adiponectin is one of the most abundant adipose tissue-specific factors and appears to improve insulin sensitivity and inhibit vascular inflammation (8,9). Serum adiponectin levels are low in obese subjects and increase after weight loss. Hypoadiponectinemia may contribute to insulin resistance and accelerated atherogenesis associated with obesity (10).

Resistin is a member of the newly discovered family of cysteine-rich secretory proteins called "resistin-like molecules" (11–13). Initial studies in rodents suggest that resistin is up-regulated in obesity, participating in the pathogenesis of insulin resistance (11). However, studies in humans have been controversial. Although some works have failed to find resistin mRNA expression in white adipose tissue of lean and obese subjects (14), others have found some expression in the white adipose tissue of obese individuals (nonmorbid and morbid), without correlation among body weight, adiposity, and insulin resistance (15). The serum levels of this protein have been scarcely reported, and there is no information about its possible variations after weight loss.

Ghrelin is a recently described peptide hormone that is secreted by endocrine cells in the gastrointestinal tract. It has been found to regulate feeding behavior by modulating expression levels of orexigenic peptides in the hypothalamus (16). Ghrelin has been implicated in the coordination of energy balance and weight regulation, and its dysregulation may be important in obesity (17,18).

Adiponectin, resistin, and ghrelin are all involved in the regulation of energy balance. They may contribute to promoting the progression of insulin resistance to type 2 diabetes and endothelial dysfunction to atherosclerosis, in the context of an increased adiposity. The complementary roles of these hormones in the regulation of adipose tissue metabolism have not been examined simultaneously in obese subjects. Moreover, the relationship between these proteins and a sustained weight loss in morbidly obese patients after gastric bypass surgery has been only partially analyzed (19–22). Hence, the aim of this study was to examine plasma adiponectin, resistin, ghrelin, leptin, and the inflammatory cytokine profile [soluble tumor necrosis factor receptors (sTNFRs) and IL-6] in nonmorbidly and morbidly obese patients. In addition, we studied these hormones before and after massive weight loss induced by gastric bypass surgery in morbidly obese patients. Because adipose tissue is an important source of proinflammatory cytokines, and adiponectin and resistin may have a modulating role in inflammation, we tested the effect of weight loss in morbidly obese patients over the proinflammatory adipokines profile, after adjusting for potential confounding factors.

Research Methods and Procedures

Morbidly Obese Subjects

Fifty-seven morbidly obese white subjects with a mean age of 42.2 ± 9.2 years (8 men and 49 women) were included in the basal study. All patients were recruited from the Endocrinology Service of the Hospital de Bellvitge (Barcelona, Spain). A gastric bypass operation was performed according to a modification of the method described by Capella and Capella (23); 34 patients completed a follow-up examination 6 months later. In brief, a small gastric upper pouch (20 mL) was created along the lesser curvature of the stomach. The stomach was stapled, and the pouch was anastomosed to a loop of jejunum. The opening between pouch and jejunum was 10 mm in diameter with a Roux-in-Y-reconstruction, whose effectiveness is related to the dumping of sweets, the satiety effect of distension of the efferent Roux limb of jejunum, and the malabsorptive effect of bypassing the distal stomach and duodenum (24). Pre- and postoperative anthropometric measurements were made, and blood samples were collected at the basal study and 24 weeks after surgery. Patients were excluded if they had had an acute major cardiovascular event in the previous 6 months, any acute illnesses and current evidence of acute or chronic inflammatory or infective diseases, or they were taking any medication that could alter lipidic or metabolic parameters. Patients receiving medical treatment for diabetes or dyslipidemia before surgery discontinued all hypoglycemic agents and hypolipemic drugs at least 3 months before basal determinations.

Nonmorbidly Obese Subjects

Consecutive nonmorbidly obese subjects (117; mean age 49.2 ± 12.4 years; 28 men and 89 women) were recruited at the Endocrinology Section of the Hospital Universitari Joan XXIII (Tarragona, Spain). None of the subjects was taking any medication or had evidence of metabolic disease other than obesity. All subjects were of white origin and reported that their body weight had been stable for at least 3 months

[1] Nonstandard abbreviations: TNF, tumor necrosis factor; IL, interleukin; WHR, waist-to-hip ratio; HOMA-IR, homeostasis model assessment of insulin resistance; HbA_{1c}, hemoglobin A_{1c}; HDLc, high-density lipoprotein cholesterol; LDLc, low-density lipoprotein cholesterol; RIA, radioimmunoassay; sTNFR, soluble tumor necrosis factor receptor.

before the study. Inclusion criteria were: BMI $>$ 27 and $<$40 kg/m^2, absence of any systemic disease, and absence of any infections in the month before the entry to the study. Liver and renal diseases were specifically excluded by biochemical work-up.

Anthropometrical Measurements

Height and weight were measured with the patient standing in light clothes and without shoes. BMI was calculated as body weight divided by height squared (kilograms per meter squared). Waist-to-hip ratio (WHR) was calculated as the ratio of waist and hip circumferences. Body composition was assessed only in morbidly obese patients by bioelectrical impedance analysis, using a Holtain BC Analyser (Cambridge, United Kingdom) (25). The precision of this test in determining body fat is within $\pm$3%. The same physician performed all examinations. Obesity was classified according to the World Health Organization criteria (26).

Analytical Methods

Blood samples were drawn from each subject before breakfast between 8 and 9 AM, after an overnight bed rest. All samples were stored at $-$80 °C until analytical measurements were performed, except for glucose, which was determined immediately after blood was drawn.

Serum glucose was measured with a glucose oxidase method using a Hitachi autoanalyzer. Serum insulin was measured using specific immunoradiometric assay (Medgenix Diagnostics, Fleunes, Belgium) in which proinsulin did not cross-react. The intra- and interassay coefficients of variation were 6% and 7%, respectively. Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated from fasting plasma insulin and glucose levels as (insulin $\times$ glucose)/22.5, where insulin concentration was reported as milliunits per liter and glucose as millimolar concentrations, which fairly correlates with the insulin sensitivity index calculated from frequently sampled intravenous glucose tolerance test minimal model analysis (27). Hemoglobin A_{1c} (HbA_{1c}) was measured by a chromatographic method (Glico Hb Quick Column Procedure, Helena Laboratories, Beaumont, TX).

Total serum cholesterol was measured through the reaction of cholesterol esterase/cholesterol oxidase/peroxidase. High-density lipoprotein cholesterol (HDLc) was quantified after precipitation with polyethylene glycol at room temperature. Serum triglycerides were measured through the reaction of glycerol-phosphatase-oxidase and peroxidase. Low-density lipoprotein cholesterol (LDLc) was calculated through the Friedewald formula (28).

Leptin concentrations were determined using a radioimmunoassay (RIA) (Linco Research), which uses human recombinant leptin for both standard and tracer, with antiserum rose against human recombinant leptin. The limit of detection was 0.5 ng/mL. The RIA for leptin does not react with human proinsulin, insulin, or glucagon.

sTNFR1 and 2 were determined by solid phase enzymeimmunoassay with amplified reactivity (Bio Source Europe). The limit of detection was 50 pg/mL for sTNFR1 and 0.1 ng/mL for sTNFR2, and the intra- and interassays were $<$7% and $<$9%, respectively. The sTNFR1 assay does not cross-react with sTNFR2. TNFα does not interfere with the assay.

IL-6 was determined by an ultrasensitive solid phase enzimoimmunoassay (Bio Source Europe, Nivelles, Belgium). The limit of the detection was 0.104 pg/mL, and intra- and interassay were 4.71 and 6.70, respectively. The kit has no cross-reactivity with IL-2, IL-4, IL-10, IL-2R, TNFα, and SCF.

Plasma Resistin, Adiponectin, and Ghrelin Concentrations

Plasma resistin levels were measured by sandwich enzyme-linked immunosorbent assay (BioVendor Laboratory Medicine, Inc., Palackeho, Czech Republic). The sensitivity was 0.2 ng/mL. The intra- and interassay coefficients of variation were 5.8% and 14.7%, respectively. Plasma adiponectin and ghrelin levels were measured using standardized RIA kits from Linco Research.

For human adiponectin, the kit has a sensitivity of 1 ng/mL in a 100-μl sample size and a range of 1 to 200 ng/mL. All samples were diluted 1/500. The intra- and interassay coefficients of variation were 8% and 12%, respectively.

For human ghrelin, the RIA kit has an antibody that is specific for total ghrelin and does not require the presence of the octonyl group on serine 3. The sensitivity was 100 pg/mL (in a 100 μL sample size) with a range of 100 to 10,000 pg/mL. The specificity of the assay was 100% for human ghrelin, ghrelin 14-28, and des-octonylghrelin. The intra- and interassay coefficients of variation were 5.63% and 16%, respectively.

Statistical Analysis

All statistical analysis were performed by using the SPSS/PC statistical program (version 10.0 for Windows; SPSS, Inc., Chicago, IL). Descriptive data were expressed as mean value $\pm$ SD or median (75th percentile). Differences among groups were compared by using a Student's *t* test or ANOVA of clinical or laboratory parameters. In morbidly obese patients, data were compared using paired Student's *t* test (pre- vs. postoperative). Variables that did not have a Gaussian distribution were logarithmically transformed. Correlation was analyzed by the Pearson product-moment correlation. Multiple linear regression analysis by forward step-wise regression was also used to analyze the independence of the association among quantitative variables.

Results

Basal Study: Nonmorbidly and Morbidly Obese Individuals

Descriptive statistics of the basal study variables are shown in Table 1. Morbidly obese subjects had higher

Table 1. Clinical and analytical characteristics in nonmorbidly and morbidly obese patients

	Non-morbid obese (n = 117, 89 women)	Morbid-obese (n = 57, 49 women)
Age (years)	49.2 ± 12.4	42.2 ± 9.2*
Weight (kg)	83.6 ± 11.1	126.9 ± 23.6†
BMI (kg/m^2)	32.9 ± 3.3	49.3 ± 7.4†
WHR	0.9 ± 0.1	0.9 ± 0.1
Fat-free mass (kg)	ND	74.2 ± 17.2
Fat mass (kg)	ND	54.5 ± 16.9
Fasting glucose (mM)	5.0 ± 0.6	7.1 ± 2.9*
Fasting Insulin (μU/mL)	15.2 ± 7.9	20.4 ± 9.0*
HbA_{1c} (%)	ND	5.6 ± 1.4
Cholesterol (mM)	5.3 ± 0.9	5.3 ± 1.1
HDLc (mM)	1.5 ± 0.4	1.1 ± 0.3*
LDLc (mM)	3.2 ± 0.8	3.5 ± 0.9*
Triglycerides (mM)	1.4 ± 0.9	2.3 ± 1.5*
HOMA-IR	3.4 ± 2.1	5.7 ± 3.9*
Leptin (ng/ml)	30.8 ± 14.7	59.7 ± 25.4*
IL-6 (pg/ml)	1.3 (1.7)	7.6 (19.4)*
sTNFR1 (ng/ml)	1.8 (2.2)	2.6 (3.3)*
sTNFR2 (ng/ml)	5.0 (5.8)	5.0 (6.5)
Ghrelin (ng/ml)	1.9 (2.4)	1.4 (2.6)
Resistin (ng/ml)	3.0 (3.8)	3.6 (5.4)*
Adiponectin (μg/ml)	16.2 (19.6)	19.6 (36.6)*

All data are presented as mean ± SD, except for IL-6, sTNFR1, sTNFR2, ghrelin, resistin, and adiponectin, which are presented as median (75th percentile). Nonparametric values were log transformed for statistical comparisons. ND, not determined.
* $p < 0.05$.
† $p < 0.01$.

values in all of the anthropometric measurements compared with nonmorbidly obese patients. The lipidic parameters showed a more atherogenic profile in morbidly obese. There were no differences in the sTNFR2 levels; however, the sTNFR1 and IL-6 levels were higher in the morbidly obese patients. Additionally, leptin, adiponectin, and resistin were significantly higher in the morbidly obese group compared with nonmorbidly obese subjects.

In the nonmorbidly obese group, gender differences were observed for leptin (13.3 ± 7.0 vs. 34.2 ± 13.4 ng/mL, $p < 0.001$) and for adiponectin (11.3 ± 4.5 vs. 18.0 ± 6.7 μg/mL, $p < 0.001$) circulating levels. Resistin and ghrelin did not show differences. Gender-related differences were not observed in morbidly obese patients in adipokine plasmatic levels.

Bivariate correlation analyses were performed to assess relationships of serum adipokines and proinflammatory cytokines with clinical anthropometric variables, body composition, and lipids in both obese groups. Pearson correlation coefficients are presented in Table 2.

In nonmorbidly obese subjects, leptin correlated positively with BMI ($r = 0.390$, $p < 0.01$), sTNFR1 levels ($r = 0.31$, $p < 0.05$), and sTNFR2 levels ($r = 0.29$, $p = 0.01$). Adiponectin showed a strong negative correlation with weight ($r = -0.33$, $p = 0.001$), triglycerides ($r = -0.22$, $p = 0.006$), and fasting insulin ($r = -0.28$, $p < 0.05$) but a positive correlation with HDLc ($r = 0.36$, $p < 0.001$). Serum resistin was positively related to sTNFR1 ($r = 0.31$, $p = 0.01$) and triglycerides ($r = 0.24$, $p = 0.01$).

In the morbidly obese group, leptin, adiponectin, and ghrelin did not correlate with any measurements of body composition. Resistin was found to correlate positively and significantly with weight ($r = 0.48$, $p < 0.001$), BMI ($r = 0.39$, $p < 0.005$), fat-free mass ($r = 0.39$, $p = 0.01$), and sTNFR2 ($r = 0.41$, $p = 0.007$). Ghrelin correlated positively only with sTNFR2 ($r = 0.45$, $p = 0.005$) (Figure 1).

Table 2. Bivariate correlation analyses in obese groups

	r	*p*
nonmorbidly obese		
Leptin		
BMI	0.39	<0.01
sTNFR1	0.31	<0.05
sTNFR2	0.29	0.01
Adiponectin		
Weight	−0.33	0.001
Triglycerides	−0.22	0.006
Fasting Insulin	−0.28	<0.05
HDLc	0.36	<0.001
Resistin		
sTNFR1	0.31	0.01
Triglycerides	0.24	0.01
Ghrelin		
Resistin	0.38	0.03
morbidly obese		
Resistin		
Weight	0.48	<0.001
BMI	0.39	<0.005
Fast-free mass	0.39	0.01
sTNFR2	0.41	0.007
Ghrelin		
sTNFR2	0.45	0.005

r, Pearson correlation coefficient.

The findings for the bivariate correlation analyses were further explored using multivariate analysis to control for potential confounders. Resistin, adiponectin, ghrelin, sTNFR1, and sTNFR2 were logarithmically transformed. After adjusting for age, gender, and BMI in nonmorbidly obese patients, leptin was positively related to gender, BMI, and sTNFR1 ($\beta = 0.52$, $p < 0.001$; $\beta = 0.24$, $p = 0.009$; and $\beta = 0.24$, $p = 0.01$, respectively). Adiponectin was positively related with HDLc and gender ($\beta = 0.31$, $p = 0.007$ and $\beta = 0.22$, $p = 0.05$, respectively) and negatively associated with weight ($\beta = -0.38$, $p < 0.001$). Resistin circulating levels were found to be positively associated with sTNFR1 after adjusting for the above-mentioned variables ($\beta = 0.28$, $p = 0.007$), losing the correlation with triglycerides observed in the bivariate analysis.

None of these adipokines was found to be associated with clinical or metabolic variables in morbidly obese subjects except for the resistin and ghrelin levels that were positively correlated with sTNFR2 after adjusting for age, gender, and BMI ($\beta = 0.41$, $p = 0.008$ and $\beta = 0.33$, $p = 0.04$ respectively).

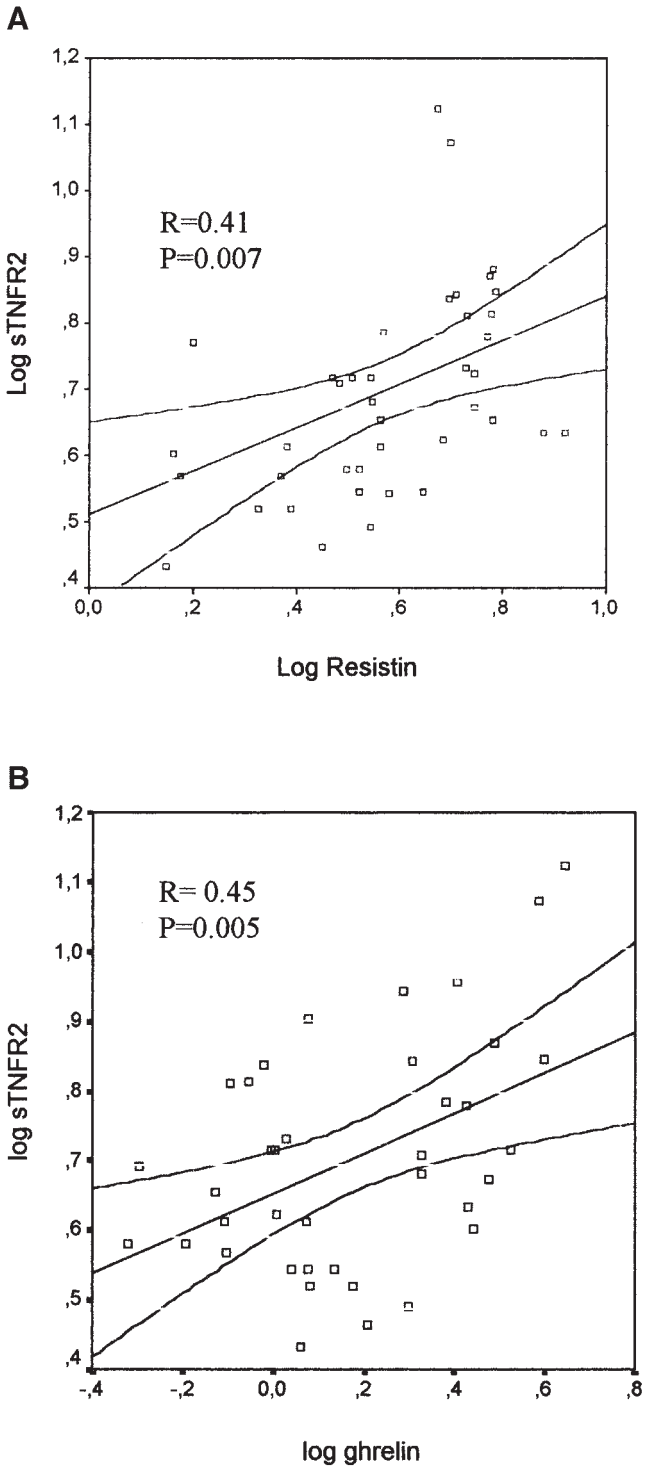

Figure 1: Correlation analysis between resistin (A) and between ghrelin (B) and sTNFR2 in morbidly obese patients before surgical procedure.

Table 3. Pre- and postoperative anthropometrical and analytical variables in surgically treated morbidly obese patients ($n = 34$)

	Preoperative	6 months
Weight (kg)	129.8 ± 24.4	91.3 ± 16.2‡
BMI (kg/m^2)	49.6 ± 5.9	34.9 ± 4.1‡
WHR	0.9 ± 0.1	0.8 ± 0.1*
Fat-free mass (kg)	73.9 ± 19.1	69.6 ± 20.5
Fat mass (kg)	57.1 ± 17.1	21.8 ± 12.1‡
Fasting glucose (mM)	7.1 ± 3.1	4.9 ± 0.9‡
Fasting insulin (μU/mL)	20.3 ± 11.2	7.3 ± 3.7‡
HOMA-IR	5.5 ± 3.5	1.5 ± 0.9‡
HbA_{1c} (%)	5.5 ± 1.2	4.7 ± 0.6‡
Cholesterol (mM)	5.1 ± 1.2	4.8 ± 0.8*
HDLc (mM)	1.1 ± 0.2	1.2 ± 0.5
LDLc (mM)	3.1 ± 0.7	2.8 ± 1.6
Triglycerides (mM)	2.4 ± 1.3	1.2 ± 0.8†

Mean values ± SD are shown for all variables.
* $p < 0.05$.
† $p < 0.01$.
‡ $p < 0.001$.

Table 4. Pre- and postoperative adipokines and proinflammatory cytokines in morbidly obese patients ($n = 34$)

	Preoperative	6 months
Leptin (μg/L)	59.6 ± 26.2	18.8 ± 9.7‡
IL-6 (pg/mL)	5.1 (18.3)	2.1 (7.2)‡
sTNFR1 (ng/mL)	2.6 (3.0)	2.2 (2.6)†
sTNFR2 (ng/mL)	4.4 (5.2)	4.4 (5.0)
Ghrelin (ng/mL)	1.1 (1.9)	1.8 (2.6)‡
Resistin (ng/mL)	3.5 (5.5)	3.4 (4.7)
Resistin-to-weight ratio	0.02 (0.01)	0.04 (0.01)†
Adiponectin (μg/mL)	20.7 (39.4)	40.9 (53.6)*

All data are presented as median (75th percentile), except for leptin and resistin-to-weight ratio, which are presented as mean ± SD. All nonparametric values were log-transformed for the statistical analysis.
* $p < 0.05$.
† $p < 0.01$.
‡ $p < 0.001$.

Follow-Up Study

Pre- and Postoperative Characteristics in Morbidly Obese Subjects

Body Composition, Plasma Lipids, and Glucose Metabolism. The gender differences before and after surgical bypass were nonsignificant; thus, data are presented collectively for both sexes. The average preoperative BMI was 49.6 ± 5.9 kg/m^2 and was dramatically reduced after surgery (20% to 39.7% weight lost). Despite massive weight loss, only one patient attained ideal body weight, and most subjects persisted with a moderate or severe obesity.

Elevated triglycerides and cholesterol levels were the most common lipid abnormalities before surgical bypass. HDLc and LDLc plasmatic levels did not show differences after weight reduction (Table 3).

Preoperative levels of plasma glucose were slightly increased, and fasting insulin and HOMA-IR were clearly elevated. Postoperatively, glucose, insulin, and HOMA-IR decreased significantly with weight loss (Table 3).

Plasma Adipokines, Ghrelin, sTNFRs, and IL-6

After bypass surgery, mean postoperative concentrations were significantly increased for adiponectin and ghrelin circulating levels. Conversely, sTNFR1, Il-6, and leptin were significantly decreased after weight loss. Resistin and sTNFR2 did not show differences 6 months after the surgical procedure (Table 4).

After adjusting for age, BMI, and sex at the end of 6 months of follow-up, leptin was positively associated with sex, sTNFR1, and BMI ($\beta = 0.54$, $p = 0.001$; $\beta = 0.39$, $p = 0.015$; and $\beta = 0.33$, $p = 0.035$, respectively). Resistin levels were positively correlated with sTNFR2 after adjusting for the above-mentioned variables ($\beta = 0.53$, $p = 0.016$). Due to the positive correlation between basal resistin levels and weight, we performed a ratio between resistin and weight to get a better understanding of the results after weight loss at the end of 6 months of follow-up. A significant increased ratio was observed after surgical bypass (Table 4).

To examine the role of the adipose tissue secreted factors as predictors of weight loss after surgical bypass, we examined by forward step-wise regression analysis the predictive value of leptin, adiponectin, and resistin in the percentage of reduction in body weight. We observed that the preoperative resistin concentration was predictive of the postoperative weight loss at 6 months of follow-up ($\beta = 0.45$, $p = 0.024$) (Figure 2); however, leptin and adiponectin did not demonstrate a significant predictive value of the extent of postsurgical weight loss. This association was maintained for the resistin-to-weight ratio (data not shown).

We also analyzed whether changes in adipose tissue hormone levels would be predictive of improved insulin sensitivity after weight loss in the morbidly obese surgically treated patients. HOMA-IR values were associated with the increase in adiponectin ($\beta = 0.77$, $p = 0.014$), whereas in this model, leptin, resistin, and BMI did not predict postoperative HOMA-IR. Additionally, step-wise regression analysis showed that the increase in percentage of change in

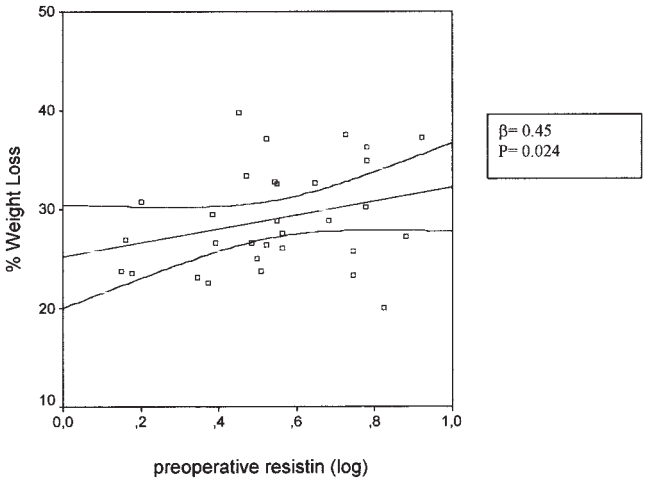

Figure 2: Regression analysis between preoperative plasma resistin concentration and percentage drop in body weight.

adiponectin significantly predicted the change in ghrelin at the end of 6 months after surgical bypass ($\beta = 0.82$, $p = 0.007$), independently of the change in weight, leptin, insulin, sTNFR1, and IL-6 levels.

Discussion

In the present study, we described a close relationship between proinflammatory cytokines and several adipokines in obese and morbidly obese patients. Massive weight loss in morbidly obese subjects caused marked changes in proinflammatory cytokines and increased the plasmatic levels of adipose tissue metabolism modulators: ghrelin and adiponectin.

TNFα system and IL6 have been implicated in the regulation of energy balance and are considered potent proinflammatory mediators, with apparent effects over many of the hormonal factors produced by the adipose tissue (18). In our study, we showed higher levels of proinflammatory cytokines (sTNFR1 and IL-6) in morbidly obese patients than in nonmorbidly obese subjects. This increased proinflammatory state was partially ameliorated after weight loss, with a concomitant reduction in both mentioned cytokines, whereas sTNFR2 levels remained high (Table 3). Different studies have been published seeking relationships between adipose tissue-derived hormones and these proinflammatory cytokines; however, there are few data, and results are controversial in morbidly obese patients.

Leptin was one of the first studied adipose tissue hormones associated with the activity of the TNF system (29,30). In vitro experiments have produced contradictory data, with an increase in leptin adipocyte production after TNFα incubation (31) or autocrine inhibition through TNFR1, in cultured adipocytes from the subcutaneous fat of pregnant women (32). However, in vivo studies are more consistent, linking the sTNFRs with leptin circulating levels in both healthy and obese subjects (29,30,33,34). We found a positive bivariate relationship between leptin and both soluble forms of TNFRs in nonmorbidly obese subjects; however, after adjusting for confounding variables, only sTNFR1 was highly correlated with circulating leptin levels. These results are in accordance with the majority of previously published data of a direct modulation of leptin secretion through sTNFR1. In fact, a recent report has demonstrated that the induction of leptin synthesis by TNFα requires activation of the TNFR1, and the activation of TNFR2 alone fails to cause leptin production (35). Another interesting aspect of our study was the observation that although these correlations were not observed in morbidly obese patients before the bypass surgery, as we had previously reported (36), after massive weight loss, leptin levels were dramatically decreased and correlated positively with BMI and sTNFR1, as in the nonmorbidly obese group. This finding suggests a recovery of the usual TNF system loop that seems to be absent in massively obese patients.

Adiponectin, a recently described adipocyte-derived hormone, has been postulated to have anti-inflammatory effects mediated, in part, by modulating TNFα activity. The suppressive effect of TNFα on adiponectin gene expression in vitro (37,38) and, conversely, the inhibition of the nuclear factor $\kappa\beta$ induced by adiponectin have been reported (39). Likewise, human tissular studies showing that subjects with the highest levels of adiponectin mRNA expression secrete the lowest levels of TNFα from their adipose tissue (40) have corroborated these findings, linking this hormone with the TNF system. However, a clear relationship between this hormone and the proinflammatory cytokine profile evaluated in our obese population was not found. Adiponectin was negatively correlated with weight and insulin in nonmorbidly obese subjects; interestingly, the basal levels of adiponectin were higher in morbidly obese patients. Although in morbidly obese patients no association was observed in the preoperative state, after weight loss adiponectin levels increased significantly and were predictive of the improvement of insulin sensitivity estimated by HOMA-IR, in agreement with a previous report (19). The absence of correlation between adiponectin and the insulin sensitivity index in the basal status supports the hypothesis that normal regulatory endocrine loops are lost in morbid obesity. Recent data have demonstrated that the insulin-sensitizing peroxisomal proliferator-activated receptor γ agonists may have a direct effect in inducing the production of adiponectin and antagonizing the effect of TNFα on the adiponectin promoter in insulin-resistant patients (41). These observations, taken together with the experimental data on the inhibitory effect of adiponectin in the TNFα synthesis, support a modulator link between both adipokines in the development of insulin resistance, although, until now, a strong correlation between them has not been found in vivo.

An interesting observation in the surgically treated morbidly obese patients was the association between the percentage of increase in adiponectin after the gastric bypass and the change in ghrelin concentration. Likewise, the ghrelin concentration in morbidly obese patients, which was lower than in the nonmorbidly obese subjects, increased after massive weight loss. This increase has been reported in at least two previous works (19,22) and is in concordance with the observations in diet-treated obese women that the increase in ghrelin levels is positively correlated with the extent of weight loss (42). A more recent work has shown that ghrelin serum concentration diminishes immediately after gastric bypass, but at 2 months follow-up, the values return to the initial levels (43). The disconnection of the stomach in surgically treated morbidly obese patients supposes, a priori, a lower stimulus for ghrelin production, with the consequent hypogrhelinemia, as has been speculated by several authors (21,44,45). Interestingly, the increased levels of ghrelin after surgical bypass indicate that ghrelin is being produced in the stomach or in a new extragastric source in these patients, suggesting different regulatory mechanisms with adiponectin, as previously proposed, in which ghrelin seems to be able to impair the gene expression of adiponectin (46).

In the present study, the level of plasma ghrelin increased in association with an increase in sTNFR2 in morbidly obese patients. There are no data reported so far of this association in massive obesity. There is only a report in cachectic patients with chronic heart failure, in which ghrelin has been positively associated with plasmatic levels of TNFα (47). The hypothesis proposed by Nagaya et al. (47), suggesting that the increased plasma ghrelin may represent a compensatory mechanism under conditions of extreme anabolic/catabolic imbalance, could help us to understand the increase in ghrelin observed after surgical bypass in the setting of a massive and fast weight loss in morbid obesity.

In contrast to previous hormones, data regarding the regulation of serum resistin levels in humans are scarce. Resistin has been proposed to play a causative role in insulin resistance through actions antagonistic to those of insulin. The majority of the studies regarding resistin regulation have been performed analyzing the mRNA adipose tissue expression in different models of rodents with insulin resistance or in adipose tissue from obese and type 2 diabetic patients (48). This is the first report, to our knowledge, that links resistin circulating levels in obesity with the TNF system. The initial experimental studies in adipose cells have suggested that TNFα suppresses resistin expression (49), which, combined with a lower expression of this protein in human adipose tissue, makes resistin a poor candidate for linking obesity and insulin resistance. However, resistin can be synthesized in peripheral blood mononuclear cells; in fact, it may be the principal source of this hormone in humans. Very recent work shows that in human peripheral blood mononuclear cells, resistin mRNA expression is strongly increased by the proinflammatory cytokines IL-1, IL-6, and TNFα, and also by lipopolysaccharides (50). In this study, we described a close association between resistin and sTNR1 in nonmorbidly obese subjects, without association with classic anthropometric or hormonal markers of obesity. Interestingly, in morbidly obese patients, this protein was positively associated with sTNFR2, BMI, and fat-free mass, suggesting a different pattern of regulation in this population. In this sense, increased levels of mRNA resistin expression have been found only in adipocytes of morbidly obese patients, in contrast with the low expression observed in obese subjects (51). In fact, we observed that there was significantly more resistin in the serum of morbidly obese subjects with no relationship to the insulin resistance index, in agreement with a very recent report (52). The majority of studies have failed to link insulin resistance with serum resistin concentrations in lean, obese, and type 2 diabetic population, suggesting a different regulatory mechanism from the one initially proposed (53–57).

On the other hand, preoperative plasma resistin concentrations were predictive of the extent of weight loss after bypass surgery. Higher preoperative resistin levels predicted a greater percentage of reduction in body weight, independently of the leptin and adiponectin levels. Resistin and sTNFR2 did not show differences in absolute values at 6 months of follow-up compared to the basal levels. However, in proportion to the decrease in body weight, the levels of both hormones were markedly increased after weight loss. One is tempted to speculate that resistin synthesis could be modulated by the proinflammatory cytokines in morbidly obese patients, acting as a buffer system from the sTNFR activity.

To conclude, TNFα receptors were involved in the regulatory endocrine system of body adiposity with an independent association with leptin and resistin axis in nonmorbidly obese patients. Our results emphasize the coordinated roles of resistin, adiponectin, and ghrelin in the modulation of the proinflammatory environment observed in obese and morbidly obese patients. Furthermore, we suggest that preoperative resistin levels are predictive of the extent of weight loss after bypass surgery in massively obese patients.

Additional studies and longer follow-up after bypass surgery are necessary to elucidate the implications of resistin in TNF activity in the etiology of metabolic disturbances associated with obesity.

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