

# Effects of Changes in Body Weight and Insulin Resistance on Inflammation and Endothelial Function in Morbid Obesity after Bariatric Surgery

Luis Alberto Vázquez, Fernando Pazos, José Ramón Berrazueta, Carlos Fernández-Escalante, María Teresa García-Unzueta, Julio Freijanes, and José Antonio Amado

*Endocrine Unit (L.A.V., F.P., M.T.G.-U., J.F., J.A.A.), Cardiology Service (J.R.B.), and Surgery Service (C.F.-E.), University Hospital Marqués de Valdecilla, University of Cantabria, Santander, Spain*

Metabolic alterations such as insulin resistance are thought to underlie the endothelial dysfunction and low grade inflammation found in morbid obesity. Twenty-six morbidly obese patients, aged  $39.0 \pm 10.0$  (mean  $\pm$  SD), were evaluated before and  $4.2 \pm 0.8$  months after bariatric surgery. A marked increment in the insulin sensitivity index ( $S_I$ ) and the endothelium-dependent vasodilatory response in a dorsal hand vein was observed after weight loss following bariatric surgery. Circulating levels of E-selectin, P-selectin, plasminogen activator inhibitor-1, and von Willebrand factor, which were higher than those in the control group, decreased significantly after surgery. Plasma vascular cell adhesion molecule-1, angiotensin-converting enzyme, intercellular adhesion molecule-1, thrombomodulin, and plasma and intraplatelet cGMP levels

did not change after weight loss. All inflammatory markers were higher in morbidly obese patients. After surgery, C-reactive protein and sialic acid diminished, whereas circulating levels of IL-6, TNF- $\alpha$ , and its soluble receptors did not. Positive correlations were found between changes in adiposity and  $S_I$  and changes in C-reactive protein and between changes in sialic acid and changes in endothelial function. In conclusion, a marked improvement in  $S_I$ , endothelial function, and low grade inflammation was observed in the weight-losing, morbidly obese patients after bariatric surgery.  $S_I$  and adiposity appear to play roles in obesity-related, low grade inflammation that contribute to the endothelial dysfunction observed in morbid obesity. (*J Clin Endocrinol Metab* 90: 316–322, 2005)

THE MASSIVE ACCUMULATION of body fat observed in morbid obesity has been associated with hyperinsulinemia and insulin resistance (IR), which appear to be the cornerstone of the obesity-related metabolic syndrome (1). Obesity and IR are associated with higher levels of circulating endothelial dysfunction biomarkers such as soluble intercellular adhesion molecule-1 (ICAM-1), soluble E-selectin (E-Sel), and von Willebrand factor (vWF) (2–6), as well as endothelial vasoactive peptides, such as angiotensinogen and angiotensin-converting enzyme (ACE) (7).

Hyperinsulinemia and IR have been involved in vascular reactivity. In the absence of IR, insulin has a direct vasodilatory effect (8, 9). However, reports assessing *in vivo* vascular reactivity in humans demonstrated that the endothelium-dependent vasodilatory response was altered in experimental mild hyperinsulinemic subjects (10) and in obese individuals who displayed IR (11, 12).

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Abbreviations: ACE, Angiotensin-converting enzyme; AUC, area under the curve; Bk, bradykinin; BMI, body mass index; CRP, C-reactive protein; E-Sel, E-selectin; F, fat mass; FSIVGTT, frequently sampled iv glucose tolerance test; ICAM-1, intercellular adhesion molecule-1; IR, insulin resistance; NE, norepinephrine; L-NMMA,  $N^G$ -monomethyl-L-arginine; NO, nitric oxide; PAI-1, plasminogen activator inhibitor-1; P-Sel, P-selectin; SA, sialic acid;  $S_C$ , glucose effectiveness at basal insulin;  $S_I$ , insulin sensitivity index; SNP, sodium nitroprusside; TB, thrombomodulin; TNFR1, TNF $\alpha$  receptor type 1; VCAM-1, vascular cell adhesion molecule-1; VD, venodilatory response; vWF, von Willebrand factor.

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In contrast, a growing body of evidence has suggested the role of low grade inflammation as a link between obesity and IR, and endothelial dysfunction. Increased serum levels of inflammatory biomarkers such as C-reactive protein (CRP) have been reported in obese subjects and have been related to the degree of insulin sensitivity and endothelial function (2, 3, 13). In fact, elevation of proinflammatory cytokines, such as TNF- $\alpha$  and IL-6, could cause IR by interfering with insulin signaling and down-regulating peroxisomal proliferator-activated receptor- $\gamma$  receptors (14, 15). Several mechanisms have been suggested to elucidate the obesity-related, low grade inflammation. Firstly, fat tissue is an important source of proinflammatory, *i.e.* TNF and IL-6, and antiinflammatory, *i.e.* adiponectin, cytokines. Secondly, IR can cause inflammation to rise by interfering with the antiinflammatory effect of insulin (16). Finally, obesity is characterized by oxidative stress, mainly from excessive macronutrient intake or increased metabolic rate, which might be partly responsible for the inflammatory response (17). Indeed, a load of lipid or glucose to obese or normal subjects leads to an increase in reactive oxygen species generation, inflammation, and lower vascular reactivity (18). Conversely, dietary restriction in obese patients leads to a significant reduction in oxidative stress and inflammation (19).

By contrast, higher TNF- $\alpha$  and IL-6 serum levels reported in morbidly obese patients decreased after weight loss in some reports (20), but not in others (21, 22). Also, the metabolic and inflammatory responses that occur in the weight-losing, morbidly obese patient after bariatric surgery are scarcely reported. In view of the fact that morbid obesity is

associated with IR, endothelial dysfunction, and low grade inflammation, we tested the hypothesis that body weight loss 4 months after bariatric surgery might improve IR, and, hence, endothelial function and inflammation. We therefore assessed insulin sensitivity by frequently sampled iv glucose tolerance test (FSIVGTT). We also measured vascular reactivity and other circulating endothelial markers to assess endothelial function. Acute phase proteins and cytokines were measured to test the innate inflammatory response. Relationships among metabolic, endothelial, and inflammatory variables were investigated to determine the mechanisms operating in the group of weight-losing, morbidly obese patients. For comparison, healthy matched control subjects were also studied

## Subjects and Methods

### Subjects

A group of 26 (23 women and three men) morbidly obese patients [age,  $39.0 \pm 10.0$  (mean  $\pm$  SD); body mass index (BMI), 46.2 (range, 36–61)] from a waiting list for bariatric surgery at University Hospital of Cantabria (Spain) were included in the study protocol. A group of 26 healthy, normal weight subjects (BMI, 23; range, 19–26), matched for age and sex, was included as the control group. A complete medical history and physical examination were performed for each subject. Subjects with diabetes mellitus, cardiovascular disease, psychiatric problems, or alcohol abuse; those receiving treatment with lipid-lowering agents, aspirin, glucocorticoids, antineoplastic agents, and bronchodilators; as well as subjects who had smoked within the last 3 months before the present study were excluded. All women were studied randomly with respect to their menstrual cycle. Each subject gave written informed consent to participate in this study, which was approved by the ethical committee of our institution.

### Study protocol

The morbidly obese patients were evaluated before (baseline) and  $4.2 \pm 0.8$  (mean  $\pm$  SD) months after surgery (fourth month). Each evaluation consisted of clinical and body composition measurements, blood sample extractions, as well as insulin sensitivity and vascular reactivity tests. Most of the basal blood chemical parameters were also determined in the control group. Two different surgical procedures were performed in obese patients; 18 patients underwent a classical vertical banded gastroplasty according to the technique proposed by Mason (23), and eight patients underwent a biliopancreatic diversion according to the technique proposed by Scopinaro (24). No significant postoperative complications occurred that prevented the patients from completing the study protocol.

### Clinical, body composition, and blood sample measurements

Every measurement was performed after an overnight fast of 12 h at the metabolic unit. A general physical examination was performed by a study physician. The systolic and diastolic blood pressure readings were recorded as the mean of two measurements with the subjects seated. Subjects' weight, height, and waist and hip circumferences were also obtained. Body fat was estimated by bioelectric impedance using a monofrequency and tetrapolar device (Bodystat 1500, Bodystat Ltd., Isle of Man, UK). Blood samples were drawn from an antecubital vein with a 19-gauge needle without venous stasis. Plasma glucose, total serum cholesterol, and triglycerides were measured using a Dax analyzer (Technicon Instruments, Tarrytown, NY). Plasma insulin was measured by RIA (ERIA Diagnostics Pasteur, Marnes la Coquette, France); C peptide was measured using an immunoradiometric assay (Diasorin, Vecellio, Italy). vWF (Asserachrom, Roche, Mannheim, Germany), plasminogen activator inhibitor-1 (PAI-1) (Innogenetics, Zwijnaarde, Belgium), E-Sel (Bender Med Systems, Vienna, Austria), soluble P-selectin (P-Sel; Bender Medical Systems), thrombomodulin (TB; Asserachrom, Diag-

nostica Stago, Asnieres-Sur-Seine, France), ICAM-1 (Bender Medical Systems), soluble vascular cell adhesion molecule-1 (VCAM-1; Bender Medical Systems), TNF- $\alpha$  (R&D Systems, Oxon, UK), soluble TNF- $\alpha$  receptor type 1 (TNFR1; R&D Systems), soluble TNFR2 (R&D Systems), and IL-6 (R&D Systems, Minneapolis, MN) were measured in plasma using an ELISA method. Intra- and interassay coefficients of variation in all assays were lower than 7% and 14%, respectively. ACE and sialic acid (SA) were measured with a Hitachi 704 analyzer (Hitachi, Tokyo, Japan) using reagents from Sigma-Aldrich Corp. (St Louis, MO) and Roche, respectively. Plasma and platelet cGMP were measured as previously described (25) using a specific enzyme immunoassay (Biotrak, Amersham Biosciences, Little Chalfont, UK). Intra- and interassay coefficients of variation were 8.7% and 11.2%, respectively. Plasma CRP levels were measured by means of immunonephelometry in a nephelometer analyzer II (Behring, Marburg, Germany) using reagents from Behring (Sommerville, NJ).

### Insulin sensitivity test

IR was assessed using Bergman's minimal model analysis based on a mathematical quantification of glucose and insulin serum levels from a 12-time point, insulin-enhanced FSIVGTT (26). This analysis provides two indicators, the insulin sensitivity index ( $S_I$ ), which is the insulin-mediated glucose disposal, and glucose effectiveness at basal insulin ( $S_G$ ), which is the glucose-mediated glucose disposal. Briefly, in the fasted state, glucose (0.3 g/kg) and insulin (0.03 U/kg) were injected iv at 0 and 20 min, respectively. Blood samples were drawn at 0, 2, 4, 8, 19, 22, 30, 40, 50, 70, 100, and 180 min for determination of glucose and insulin serum levels. The area under the curve for insulin in response to iv glucose was determined between 0 and 19 min ( $AUC-I_{0-19}$ ) using the trapezoidal rule of each FSIVGTT.

### Vascular reactivity test

To assess endothelial vasodilatory function, the vascular reactivity of one of the dorsal hand veins, measured by a linear differential transformer technique before and after bradykinin (Bk; endothelium-dependent nitric oxide (NO)-mediated venodilation) and sodium nitroprusside (SNP) (endothelium-independent NO-mediated venodilation) infusion, was determined as previously described (27). Briefly, the study was carried out in a temperature-controlled room (28–30 C), and an overnight fast was required. A 23-gauge butterfly needle was inserted into a preselected vein on the back of the hand. A tripod holding a linear variable differential transformer was attached to the perivenous surface. The linear transformer signaling records stood for variations in venous diameter. All records were taken under a congestive pressure of 40 mm Hg. The saline-induced venous diameter was considered 100% venodilation. Thereafter, increasing norepinephrine (NE) doses were infused to achieve a progressive venous constriction. The NE dose producing 50% reduction in venous diameter (0% venodilation) was administered at a fixed flow rate throughout the test. Both the Bk- (Bk-VD) and SNP-induced (SNP-VD) venodilatory responses were depicted as the percent venous diameter change from NE-induced (0%) venodilation. The inhibitory effect of N<sup>G</sup>-monomethyl-L-arginine (L-NMMA) on Bk-VD was also shown.

### Statistical analysis

Data are presented as the mean  $\pm$  SEM. For parametrically distributed data, comparisons were made using the paired *t* test for data within the obese group and ANOVA followed by Bonferroni *post hoc* test for data between groups. For nonparametrically distributed data, Wilcoxon and Kruskal-Wallis tests were used where appropriate. Correlations between changes in the variables were tested using univariate analyses (Pearson's or Spearman's correlation where appropriate).  $P < 0.05$  was considered statistically significant.

## Results

### Clinical and metabolic effects

The effect of bariatric surgery on clinical, anthropometric, and adiposity measurements are shown in Table 1. Body

**TABLE 1.** Clinical measurements and indices of sensitivity and secretion of insulin in morbidly obese patients before (Baseline) and after (4th month) bariatric surgery

	Baseline	4th month
Weight (kg)	126.3 ± 5.7	99.6 ± 4.3 <sup>a</sup>
BMI (kg/m <sup>2</sup> )	46.2 ± 1.3	36.7 ± 1.2 <sup>a</sup>
Waist circumference (cm)	127.9 ± 3.4	115.1 ± 3.1 <sup>a</sup>
Hip circumference (cm)	133.9 ± 2.6	119.9 ± 2.2 <sup>a</sup>
F (kg)	63.9 ± 3.3	42.4 ± 2.5 <sup>a</sup>
Systolic blood pressure (mm Hg)	134.8 ± 3.8	128.1 ± 3.2 <sup>b</sup>
Diastolic blood pressure (mm Hg)	91.4 ± 4.0	86.0 ± 2.4 <sup>b</sup>
Total cholesterol (mmol/liter)	5.0 ± 0.2	4.3 ± 0.2 <sup>c</sup>
Triglycerides (mmol/liter)	1.4 ± 0.1	1.0 ± 0.1 <sup>a</sup>
S <sub>I</sub> (×10 <sup>-4</sup> /min <sup>1</sup> ·μU/ml)	0.87 ± 0.16	2.38 ± 0.29 <sup>a</sup>
S <sub>G</sub> (×10 <sup>-2</sup> /min)	2.20 ± 0.36	1.86 ± 0.22
C Peptide (μg/liter)	4.0 ± 0.3	2.1 ± 0.2 <sup>a</sup>
AUC-I <sub>0-19'</sub> (μIU/ml)	1399.7 ± 203.2	785.6 ± 110.2 <sup>a</sup>

Data are the mean ± SEM. AUC-I<sub>0-19'</sub>, Area under curve for insulin. Insulin conversion factor (CF) to picomoles per liter, 7.175.

<sup>a</sup> *P* < 0.001 vs. baseline.

<sup>b</sup> *P* < 0.05 vs. baseline.

<sup>c</sup> *P* < 0.01 vs. baseline.

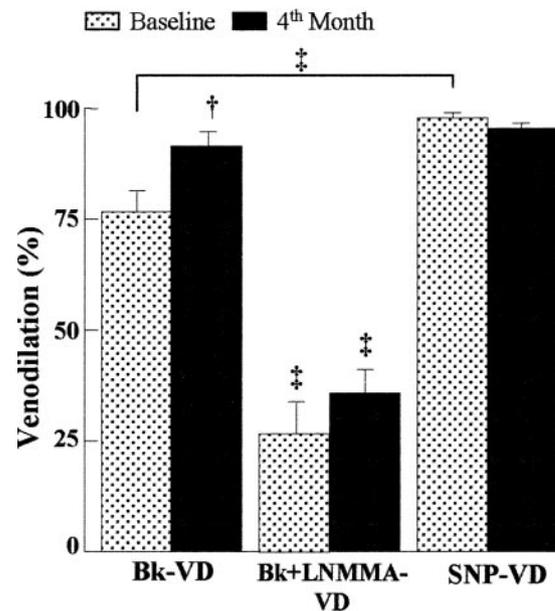
weight, BMI, waist circumference, hip circumference, and fat mass (F) decreased markedly 4 months after surgery. S<sub>I</sub> was markedly increased, even though S<sub>G</sub> remained unchanged. Baseline C peptide, a marker of endogenous insulin secretion, and AUC-I<sub>0-19'</sub>, indicating the insulin response to the glucose challenge during FSIVGTT, were also decreased significantly (Table 1).

#### Vascular reactivity testing

The Bk-VD significantly increased 4 months after surgery compared with that at baseline (76.76 ± 4.71% vs. 91.74 ± 2.90%; *P* < 0.01). This response was markedly inhibited by concomitant administration of the NO production inhibitor l-NMMA at both times (65.94 ± 7.08% vs. 60.80 ± 5.44%; not significant). Furthermore, SNP-VD (endothelium-independent), which was higher than the Bk-VD at baseline, remained unchanged after surgery (97.87 ± 1.06% vs. 95.83 ± 1.61%; not significant). By the fourth month after surgery, no differences between Bk-VD and SNP-VD were found (Fig. 1).

#### Endothelial and inflammatory biomarkers

Table 2 shows the circulating endothelial and inflammatory mediators before and after surgery in morbidly obese patients. These values were compared with the data obtained from the control group. ACE levels were not different among groups. vWF levels decreased after surgery, but remained significantly higher than those in the control group. E-Sel levels decreased after surgery to the levels found in the control group. P-Sel levels decreased significantly after bariatric surgery in the obese group. ICAM-1 levels remained unchanged among groups. VCAM-1 levels were higher in obese patients after surgery compared with those in the baseline and control groups; no differences at baseline between groups were found. TB remained unchanged among groups. PAI-1 levels, which were higher at baseline in obese patients, decreased after surgery to values similar to those in the control group. Plasma cGMP levels were lower in patients with morbid obesity than in the control group and



**FIG. 1.** Vascular reactivity testing in morbidly obese patients. Dorsal hand vein dilations in response to Bk (Bk-VD), Bk with l-NMMA (Bk+l-NMMA-VD), and SNP (SNP-VD) were measured using a linear differential transformer technique in the same patients before (baseline; ▨) and after bariatric surgery (fourth month; ■). The vasodilatory effects were expressed as the percent dilation between 0 and 100% obtained by NE and normal saline infusions, respectively. Data are the mean ± SEM. †, *P* < 0.01 vs. baseline; ‡, *P* < 0.001 vs. Bk-VD.

remained unchanged after surgery. Platelet cGMP levels remained unchanged among groups. SA levels, which were higher at baseline in the obese group, decreased to values similar to those in the control group. TNF-α levels were higher in obese patients and remained unchanged after bariatric surgery. TNFR1 levels were higher in obese patients, and a nonsignificant decrease after bariatric surgery was observed. TNFR2 levels were higher in obese patients and increased after bariatric surgery. IL-6 levels were higher in obese patients, but remained unchanged after bariatric surgery. CRP levels were higher in obese patients and decreased after surgery to values higher than those in the control group. Circulating SA levels, which were higher in the obese group, decreased after surgery to levels similar to those in the control group.

#### Correlational analysis in morbid obesity

To examine the mechanisms of the marked weight loss following bariatric surgery on endothelial function and inflammation, we examined correlations among changes in indexes of insulin action, changes in indexes of adiposity, changes in endothelium-dependent vasodilation, and changes in endothelial and inflammatory markers (Table 3). Significant correlations among the anthropometric and adiposity measurements were found between changes in S<sub>I</sub> and BMI, and between changes in waist circumference and F. Also, changes in S<sub>I</sub> were found to be correlated with changes in TB and strongly with changes in CRP among endothelial and inflammatory markers, respectively. The only relationship of changes in Bk-VD that we found was with changes in SA, which suggested inflammation to impair endothelium-

**TABLE 2.** Circulating levels of both endothelial and inflammatory biomarkers in morbidly obese patients before (Baseline) and after bariatric surgery (4th month) compared with a normative values of these mediators in a healthy, pairwise-matched, control group

Variables	Obese baseline	Obese 4th month	Control baseline
ACE (U/liter)	34.76 ± 4.06	30.50 ± 3.29	40.53 ± 3.15
vWF (%)	82.33 ± 6.16	72.94 ± 7.54 <sup>a</sup>	29.99 ± 3.15 <sup>b,c</sup>
Soluble E-Sel (ng/ml)	45.31 ± 6.17	24.92 ± 2.88 <sup>b</sup>	23.49 ± 2.50 <sup>b</sup>
Soluble P-Sel (ng/ml)	112.58 ± 14.35	78.70 ± 7.84 <sup>b</sup>	96.70 ± 7.06
ICAM-1 (ng/ml)	206.27 ± 13.65	202.16 ± 11.43	182.08 ± 9.71
VCAM-1 (ng/ml)	1012.10 ± 50.40	1268.84 ± 61.98 <sup>b</sup>	937.28 ± 51.50 <sup>c</sup>
TB (ng/ml)	29.28 ± 2.23	30.58 ± 1.93	29.50 ± 2.34
PAI-1 (ng/ml)	161.08 ± 9.11	98.24 ± 8.45 <sup>b</sup>	77.32 ± 6.12 <sup>b</sup>
cGMP (plasma; pg/ml)	4.35 ± 0.24	4.36 ± 0.30	9.38 ± 1.34 <sup>b,c</sup>
cGMP (platelet; pg/ml)	1.27 ± 0.09	1.28 ± 0.11	0.89 ± 0.14
Sialic acid (mg/dl)	89.65 ± 2.46	83.53 ± 2.30 <sup>a</sup>	80.18 ± 2.10 <sup>a</sup>
TNF $\alpha$ (pg/ml)	2.88 ± 0.22	2.72 ± 0.22	1.35 ± 0.21 <sup>b,c</sup>
TNFR1 (pg/ml)	1378.99 ± 49.39	1279.53 ± 51.31	1111.61 ± 45.75 <sup>b</sup>
TNFR2 (pg/ml)	2329.76 ± 79.14	2572.35 ± 96.43 <sup>a</sup>	1979.73 ± 74.45 <sup>b,c</sup>
IL-6 (pg/ml)	4.93 ± 0.70	4.92 ± 0.69	3.17 ± 0.91 <sup>b,c</sup>
CRP (mg/dl)	0.63 ± 0.10	0.40 ± 0.06 <sup>a</sup>	0.15 ± 0.04 <sup>b,c</sup>

Data are the mean ± SEM.

<sup>a</sup>  $P < 0.05$  vs. baseline (obese).

<sup>b</sup>  $P < 0.004$  vs. baseline (obese).

<sup>c</sup>  $P < 0.001$  vs. 4th month (obese).

dependent venodilation. Amongst the changes in circulating endothelial markers, the largest were between ICAM-1 and TNFR2, VCAM and TNFR2, vWF and SA, E-Sel and SA, E-Sel and TNFR2, TB and SA, ACE and SA, and PAI-1 and F. Most of the changes in inflammatory markers were correlated with each other. The results of both correlational and mean difference analyses in the group of patients that underwent vertical banded gastroplasty surgery were similar to those in the bilious-pancreatic diversion group, even though several significant associations were lost in the correlational analysis as the number of patients decreased.

## Discussion

The present study confirms that in morbidly obese, non-diabetic patients, a marked weight loss 4 months after bariatric surgery results in a marked reduction in both IR and basal and stimulated insulin secretion. In agreement with previous reports,  $S_I$  increased, insulin secretion decreased, and  $S_G$  remained unchanged, suggesting a major role for insulin-mediated mechanisms in the pathogenesis of IR in morbid obesity (28). In addition to reduction in IR, this marked weight loss has been found to be associated with improvement in the clustering of obesity-related metabolic

**TABLE 3.** Correlations of changes among body composition measurements, indices of both sensitivity and secretion of insulin, circulating endothelial markers, and inflammatory changes and inflammatory markers

	$\Delta F$	$\Delta SI$	$\Delta Bk-VD$	$\Delta TNF\alpha$	$\Delta TNFR1$	$\Delta TNFR2$	$\Delta IL-6$	$\Delta CRP$	$\Delta SA$
$\Delta BMI$	0.90 <sup>a</sup>	-0.45 <sup>b</sup>	0.06	0.23	-0.11	0.01	-0.31	0.35	-0.08
$\Delta F$			-0.07	0.21	-0.13	-0.01	-0.25	0.44 <sup>b</sup>	-0.05
$\Delta WC$	0.42 <sup>b</sup>	-0.61 <sup>c</sup>	0.19	0.25	-0.06	0.35	-0.26	0.01	-0.15
$\Delta SI$	-0.47 <sup>b</sup>		-0.22	0.25	0.14	0.12	-0.01	-0.60 <sup>c</sup>	-0.18
$\Delta AUC1$	-0.01	0.29	0.16	0.20	-0.06	0.14	-0.11	-0.41 <sup>b</sup>	-0.24
$\Delta Bk-VD$	-0.07	-0.22		0.05	0.07	-0.07	0.00	0.16	-0.45 <sup>b</sup>
$\Delta ICAM-1$	0.17	0.25	0.06	0.15	0.22	0.49 <sup>c</sup>	0.41 <sup>b</sup>	0.34	0.28
$\Delta VCAM$	-0.04	0.38	-0.09	0.21	0.25	0.48 <sup>c</sup>	0.36	0.05	0.35
$\Delta PAI-1$	0.48 <sup>c</sup>	-0.22	-0.05	0.23	-0.06	-0.22	-0.16	0.35	-0.11
$\Delta vWF$	-0.05	-0.10	-0.18	-0.16	-0.19	0.28	-0.17	-0.12	0.44 <sup>b</sup>
$\Delta E-Sel$	0.28	-0.33	-0.24	0.18	0.12	0.42 <sup>b</sup>	-0.01	0.30	0.49 <sup>c</sup>
$\Delta P-Sel$	0.15	0.11	-0.17	0.07	0.08	-0.06	-0.04	-0.05	-0.05
$\Delta TB$	-0.18	0.45 <sup>b</sup>	-0.13	0.00	0.26	0.04	0.21	-0.03	0.47 <sup>b</sup>
$\Delta ACE$	0.02	-0.09	-0.05	0.35	0.35	0.36	0.38	0.35	0.46 <sup>b</sup>
$\Delta TNF\alpha$	0.21	0.25							
$\Delta TNFR1$	-0.13	0.14		0.58 <sup>c</sup>					
$\Delta TNFR2$	-0.01	0.12		0.36	0.54 <sup>c</sup>				
$\Delta IL-6$	-0.25	-0.01		0.06	0.52 <sup>c</sup>	0.51 <sup>c</sup>			
$\Delta CRP$	0.44 <sup>b</sup>	-0.60 <sup>c</sup>		0.33	0.49 <sup>c</sup>	0.45 <sup>b</sup>	0.38 <sup>b</sup>		
$\Delta SA$	-0.05	-0.18		0.13	0.45 <sup>b</sup>	0.52 <sup>c</sup>	0.70 <sup>a</sup>	0.56 <sup>c</sup>	

Data are correlation coefficients.  $\Delta$ , Changes from basal to 4th month.  $AUC_{10-19}$ , Area under curve for insulin; WC, waist circumference.

<sup>a</sup>  $P < 0.001$ .

<sup>b</sup>  $P < 0.05$ .

<sup>c</sup>  $P < 0.01$ .

syndrome, blood pressure, triglycerides, and cholesterol. These findings make our model suitable to study the mechanisms by which the improvement in obesity-related metabolic syndrome might modify cardiovascular risk.

The present study demonstrated that in morbid obesity, venous vasodilatory function induced by Bk, an endothelium-dependent vasodilator (29), improved after bariatric surgery, and NO production and NO synthase activity are involved in the improvement in vascular reactivity. Resistance to the vasodilatory effect of insulin (30), inflammatory cytokines (31), and the obesity-related increased formation of reactive oxygen species, including superoxide (18), have been reported to play a major role in the impairment of obesity-related (12) endothelial NO-mediated vasodilation. In this group with morbid obesity, the correlation analysis among changes in Bk-VD and SA suggests that over the medium term, low grade inflammation may be the link between obesity-related IR and vasodilatory function.

In addition to vasodilatory function, we studied several circulating endothelial biochemical markers. This study shows that in nondiabetic, morbidly obese patients, levels of most of the endothelial dysfunction biomarkers are higher than those in control lean subjects. These chemical mediators that arise from endothelial cells in response to inflammation or damage, regulating the attachment and transmigration of leukocytes across endothelial line, are likely to play a role in the development and/or progression of atherosclerosis, and they can be used clinically as markers of cardiovascular events (32). In agreement with previous reports, the higher levels of P-Sel decrease after bariatric surgery (33). We also found, for the first time to our knowledge, that levels of E-Sel and vWF decrease after weight loss in morbid obesity. The observation of increased circulating cellular adhesion molecules and their relationships with the inflammatory markers led us to suggest endothelial activation as a link between obesity and atherosclerosis. In contrast, even though no differences were found between the obese patients and the control group at baseline, VCAM increased slightly. Higher plasma VCAM-1 levels have been reported in obesity by some researchers, but not by others (32, 33), and some controversy exists over the clinical significance of circulating VCAM-1 to vascular damage (34). This study failed to show any change in ACE, ICAM-1, platelet cGMP, and TB between groups. Changes in these molecules have been found in relation to moderate obesity or diabetes-related IR (7, 33, 35, 36). Differences in the experimental models, *i.e.* moderate *vs.* severe obesity, may explain these discrepancies. The endothelium-derived molecule involved in vascular hemostasis, PAI-1, is also expressed in adipose tissue and is related to obesity and IR (37). In the morbidly obese group, weight loss after bariatric surgery normalized the augmented plasma PAI-1 levels in accordance with previous reports (38). Furthermore, the PAI-1 changes were strongly correlated with measurements of adiposity. These findings suggest that body weight loss improves, at least partially, the prothrombotic tendency observed in severe obesity.

This study shows that the low grade inflammation improved due to the weight-losing effect of bariatric surgery, and this improvement has been strongly related to insulin sensitivity and adiposity. According to previous reports, el-

evation of both the acute phase mediators, CRP and SA, and the proinflammatory cytokines, TNF and IL-6, has characterized morbid obesity as a low grade inflammatory process of the innate immune system (39, 40). The strong relationship between changes in S<sub>1</sub> and adiposity with changes in CRP in the morbidly obese group suggests that the state of IR results, at least partly, in the development of inflammation by interfering with the antiinflammatory effect of insulin (16). Also, it suggests that adiposity, which is characterized by oxidative stress, mainly from excessive macronutrient intake or increased metabolic rate, might induce inflammation by activation of the redox-sensitive proinflammatory transcription factor, nuclear factor- $\kappa$ B (41). Indeed, administration of a load of lipid or glucose to obese or normal subjects leads to an increase in reactive oxygen species generation, inflammation, and lower vascular reactivity (18); in contrast, dietary restriction in obese patients leads to a significant reduction in oxidative stress and inflammation (19). Alternatively, fat tissue is an important source of proinflammatory, *i.e.* TNF and IL-6, and antiinflammatory, *i.e.* adiponectin, cytokines, which, in turn, might result in IR (42). In contrast, this study failed to show any change in TNF- $\alpha$  and IL-6 4 months after surgery, whereas a nonsignificant decrease in TNFR1 and a slight increase in TNFR2 serum levels were observed. Although these findings could not be explained by the findings of this study, it appears that inflammatory pathways involving the TNF- $\alpha$  system and IL-6 remain activated. In obese patients, it has been stated that body weight loss induced by a low calorie diet and behavior modifications lower circulating TNF- $\alpha$  and IL-6 levels (20, 33). In contrast, the absence of changes in these inflammatory cytokines has been recently reported in very active, weight-losing patients induced by a very low calorie diet (43) or bariatric surgery (21, 22), suggesting a role of the metabolic stress of relative starvation in the inflammatory response (44). Alternatively, due to the fact that our patients remained relatively obese, it might be hypothesized that a certain amount of adiposity must be lost before any effect on these cytokines is observed. Additional studies in different human weight-losing models are required to elucidate the mechanisms underlying the effect of body weight reduction on inflammatory pathways that involve TNF- $\alpha$  and IL-6.

Although the physiological significance of plasma levels of the soluble fraction of TNF- $\alpha$  receptors is not fully understood, it has been previously described their relationship with obesity (45). We found that TNF- $\alpha$  receptors were positively related to adiposity and endothelial dysfunction markers. Moreover, the correlation between the increases in VCAM-1 and TNFR2 levels after surgery suggests that the persistence in the activation of this low grade inflammation pathway might be involved in the lack of decrease in VCAM-1 levels that we observed.

Our findings showed the associations of endothelial function and adiposity with CRP and SA to be apparently heterogeneous. However, the correlational analysis of changes in these inflammatory markers showed that they were closely related to each other throughout this study. Also, discordance between the plasma concentrations of different acute phase proteins is relatively common, because they are individually regulated and have different patterns of pro-

duction (46). Furthermore, blood SA levels reflect an integrated measure of the acute phase response, because many of the acute phase proteins are glycoproteins with SA at the terminal of the oligosaccharide chain (47), which might be modified by variations in blood levels of most acute phase proteins as well as their posttranscriptional sialylation. Recent reports have indicated the difficulty of characterizing this low grade inflammatory state on the basis of a single marker, suggesting the use of a summary measure of several markers as an inflammatory score to better realize the inflammatory state (48). Finally, because the possible correlation between venous and arterial dysfunction remains to be demonstrated in morbid obesity, discrepancies with previous reports in the associations between inflammatory markers and vasodilatory response (49, 50) may be related to different study protocols, *i.e.* venous *vs.* invasive and non-invasive arterial models (29).

Taken together, these findings show that in morbidly obese patients after bariatric surgery, weight loss, insulin sensitivity, endothelial function, as well inflammatory response improve in parallel over the medium term. Changes in IR and obesity status appear to be involved in the decrease in the inflammatory response, although IL-6 and TNF- $\alpha$  systems remained activated 4 months after bariatric surgery. These findings suggest that metabolic events and mediators other than TNF- $\alpha$  and IL-6, such as insulin and oxidative stress, seem to play a role in serum levels of CRP and SA in our model of morbid obesity. Throughout this study, E-Sel and PAI-1, and CRP and SA have been shown to be the earliest and most consistent markers of endothelial dysfunction and inflammatory response, respectively. The practical utility of these markers in cardiovascular risk evaluation and in the results of interventional procedures in morbid obesity should be considered.

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Address all correspondence and requests for reprints to: Dr. Fernando Pazos, Endocrine Unit, P.O. Box 2257, 39080 Santander, Spain. E-mail: endptf@humv.es.

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