

Soluble CD40 Ligand in Morbidly Obese Patients

Effect of Body Mass Index on Recovery to Normal Levels After Gastric Bypass Surgery

Juan A. Baena-Fustegueras, MD; Eva Pardina, PhD; Eva Balada, MD; Roser Ferrer; Roberto Catalán, PhD; Joaquín Rivero, MD; Isidre Casals, PhD; Albert Lecube, MD; Jose M. Fort, MD; Víctor Vargas, MD; Julia Peinado-Onsurbe, PhD

Importance: In recent years, the CD40/CD40L system has been implicated in the pathophysiology of severe chronic inflammatory diseases. Recently, obesity has been described as a low chronic inflammatory disease, so this system could also be involved in the inflammatory process.

Objective: To study soluble CD40 ligand (sCD40L) and other factors implicated in coagulation (plasminogen activator inhibitor 1, antithrombin III, and fibrinogen) and inflammation (C-reactive protein) in patients with morbid obesity and different body mass indexes (BMIs) (calculated as weight in kilograms divided by height in meters squared), before and after weight loss induced by bariatric surgery.

Design: Plasma samples were obtained before and after a bariatric surgery intervention. Several inflammatory markers were then studied (sCD40L, plasminogen activator inhibitor 1, antithrombin III, and C-reactive protein). The values obtained were compared with a control group of nonobese persons.

Participants: Thirty-four morbidly obese patients undergoing gastric bypass surgery and 22 normal-weight controls matched for age and sex.

Interventions: A Roux-en-Y gastric bypass was performed in morbidly obese patients.

Main Outcome Measures: Levels of sCD40L, plasminogen activator inhibitor 1, antithrombin III, fibrinogen, and C-reactive protein 12 months after bariatric surgery.

Results: Obese men showed a tendency for decreased plasma sCD40L levels 1 year after surgery (mean [SEM], 246.5 [70.4] pg/mL before vs 82.2 [23.2] pg/mL after surgery; $P < .05$), whereas there were not any significant changes in obese women (285.9 [67.5] pg/mL before vs 287.0 [56.9] pg/mL after surgery). Levels of the other markers studied decreased significantly with weight loss in both sexes. However, all other studied markers tend to have higher concentrations in patients with higher BMIs, except for sCD40L, which tended to have lower concentrations in patients with BMIs higher than 55. The decreases with weight loss were lower with higher BMIs for all measurements, except for antithrombin III.

Conclusions and Relevance: Increased BMI, but not sex, influences recovery to normal levels for the markers studied, possibly indicating a worse prognosis.

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IN RECENT YEARS, THE CD40/CD40 ligand (CD40L) system has been implicated in the pathophysiology of severe chronic inflammatory diseases, including atherosclerosis.^{1,2} Increasing evidence implicates CD40L as a central signaling mechanism that stimulates an array of proatherothrombotic processes.^{3,4} The CD40 ligation leads to activation of inflammatory cells, endothelium, and platelets. These cells are integral to the development of plaque disruption, acute coronary syndromes, and recurrent cardiovascular events. The CD40L is rapidly upregulated during platelet activation and triggers an inflammatory response in cells that constitutively express CD40, that is, endothelial cells and mono-

cytes.⁵ Both CD40 and CD40L are overexpressed in human and experimental atherosclerotic lesions, particularly in advanced, rupture-prone plaques.⁶ Conversely, CD40L induces tissue factor expression,⁷ accounting for thrombotic events within the plaque. Additional evidence suggesting a link between atherosclerosis and the inflammatory properties of CD40/CD40L has emerged from several prospective studies.^{8,9} Studies in patients with acute coronary syndromes revealed that elevated soluble CD40L (sCD40L) levels indicated a significantly increased risk of death or nonfatal myocardial infarction.¹⁰ Elevated plasma concentrations of sCD40L were observed in patients with unstable angina¹¹ and were predictive for high-risk atheroscle-

Author Affiliations are listed at the end of this article.

rotic lesions⁸ and the risk for future cardiovascular events in women.¹²

Android obesity is associated with enhanced lipid peroxidation and persistent platelet activation, both of which can be improved by successful weight loss.¹¹ These abnormalities are driven by inflammatory triggers related to the degree of abdominal adiposity. Santilli et al¹¹ found significantly higher plasma C-reactive protein (CRP) levels associated with enhanced oxidative stress and platelet activation in otherwise healthy women with visceral obesity. Others showed increased CD40L levels in patients with severe obesity¹² and demonstrated that weight loss during a 16-week period of caloric restriction was associated with significant reductions in sCD40L levels and oxidative stress.¹³

Because morbid obesity is closely associated with chronic inflammation and thrombotic events, our goal was to study levels of sCD40L and other biomarkers, such as CRP, antithrombin III (ATIII), and plasminogen activator inhibitor 1 (PAI-1), in patients with morbid obesity and different body mass indexes (BMIs) (calculated as weight in kilograms divided by height in meters squared), before and after weight loss induced by bariatric surgery.

METHODS

PATIENT SELECTION

A group of 34 morbidly obese patients (24 women and 10 men), aged 27 to 61 years, were recruited from the Vall d'Hebron Hospital in Barcelona, Spain. Our specific inclusion criteria were as follows: a BMI higher than 40 or a BMI between 35 and 40 and at least 2 comorbid conditions (eg, diabetes, dyslipidemia, and steatosis), stable weight during the previous 3 months, and no insulin treatment in diabetic patients. The diagnostic criteria (ie, fasting glucose level >110 mg/dL [to convert glucose to millimoles per liter, multiply by 0.0555], waist circumference larger than 102 cm in men and 88 cm in women, triacylglyceride levels >150 mg/dL [to convert triacylglycerides to millimoles per liter, multiply by 0.0113], high-density lipoprotein cholesterol level <40 mg/dL in men and 50 mg/dL in women [to convert cholesterol to millimoles per liter, multiply by 0.0259], and hypertension >130/83 mm Hg) that we used for diabetes, hypertension, and metabolic syndrome are those proposed by the National Cholesterol Education Program.¹⁴ Twenty-three (68%) of the patients had metabolic syndrome. All participants were free of inflammatory disease (other than obesity) and infectious diseases, and none were receiving antiobesity or anti-inflammatory drugs at the time of the study. Patients were excluded if they had neoplastic, renal, or active systemic diseases; hypothyroidism; or an endocrine disease other than diabetes. Height, weight, and waist and hip circumferences were recorded with participants wearing light clothing and no shoes. Body mass index was calculated. Waist circumference was measured at the natural indentation between the 10th rib and the iliac crest (minimum waist circumference). Hip circumference was measured over the widest part of the gluteal region.

The participants were divided into 4 groups depending on their BMI: group 1, BMI lower than 45 (n=8); group 2, 45 to 49.9 (n=10); group 3, 50 to 54.9 (n=12); and group 4, 55 to 60 (n=4). Obesity was defined as a BMI higher than 30. Twenty-two normal-weight persons (BMI <25; matched in age and sex with patients in the obese group), volunteer blood donors from a hospital clinic blood bank, were used as controls; they were euthyroid, normolipemic, and without digestive system diseases. The study proto-

col was reviewed and accepted by the hospital ethics committee, and all participants (controls and obese patients) gave their written informed consent to participate.

BLOOD SAMPLES AND ASSAY

Patients' blood samples were obtained after an overnight fast before and 12 months after surgery. All obese patients underwent an open Roux-en-Y gastric bypass using the Fobi-Capella technique (ring, 7 cm; Roux-limb, 180 cm; biliopancreatic limb, 80 cm). The gastric reservoir was tested with a 36F Foucher probe. The foot loop was performed by side-to-side anastomoses.

Plasma was separated immediately by centrifugation (2000g for 30 minutes at 4°C), and aliquots were frozen at -80°C for subsequent measurement of biochemical and inflammatory factors. In the control group, a blood sample was obtained after an overnight fast using minimal tourniquet pressure. All the plasma samples were stored at -80°C until assay.

Fasting plasma glucose was measured enzymatically by the hospital's routine chemistry laboratory. Insulin was measured with an autoanalyzer (IMMULITE 2500; Siemens Medical Diagnostics). The measurements were based on a noncompetitive chemiluminescent immunometric assay with 2 binding sites in solid phase. The homeostatic model assessment of insulin resistance was calculated as described elsewhere by Matthews et al.¹⁵

Plasma concentrations were determined as follows: sCD40L was measured with an enzyme-linked immunosorbent assay (ELISA; ELISA Quantikine sCD40L; R&D Systems); PAI-1, a measure of impaired fibrinolysis, with ELISA (IMUBIND; America Diagnostica); ATIII, with the chromogenic anti-Xa method (BIOPEP SA); and CRP, with a turbidimetric assay (Gerron reagents; RAL). Plasma blood cell and platelet counts were measured with an automatic analyzer (Beckman Coulter LH 750; Beckman Coulter), homeostasis and blood coagulation were measured with an automatic autoanalyzer (Amelung CS-400; Grifols), and calcium and iron were measured with an automatic autoanalyzer (Olympus AU 5400; Olympus).

STATISTICAL ANALYSIS

Results are given as means (SEMs). Differences between mean values in obese patients before and 12 months after surgery were analyzed with a 2-tailed paired *t* test. When obese and normal-weight participants were compared, significance was assessed using an unpaired 2-tailed *t* test. Statistical differences between mean values for obese or 12-month postoperative patients by sex and BMI were assessed with 2-way analysis of variance. Individual comparisons were made with the Bonferroni multiple-comparison test. All statistical analyses were computed with the GraphPad Prism software program, version 5.00 for Windows (GraphPad Software).

RESULTS

Clinical characteristics before and 12 months after surgery are shown in **Table 1**. All markers measured, except platelets (counts and volume) and iron levels, were significantly decreased 1 year after surgery.

EFFECTS OF SEX AND WEIGHT LOSS ON MARKERS OF COAGULATION AND INFLAMMATION

The coagulation and inflammation markers measured in control, obese, and 12-month postoperative groups are

summarized in **Table 2**. All measurements were significantly lower in controls than in the obese group. Notably, the mean sCD40L level in controls was 126 times lower than in obese patients. Considering both sexes together, weight loss seems slightly associated with a decline in sCD40L levels, but this decline was not significant because of the wide dispersion of the data. For men considered separately, there was a clear, marginally significant decrease in sCD40L levels (3-fold, $P < .05$); no significant changes were observed in women (Table 2).

The PAI-1 values were significantly higher in obese patients than in controls. However, in all the patients with weight loss, the postoperative values were lower than the control values (39% lower in men and 58% lower in women). The ATIII values were significantly lower in controls than in obese patients, but, curiously, these values increased further with weight loss. Both fibrinogen and CRP levels were higher in obese patients than in controls, and as observed for PAI-1, the trend was for them to decrease significantly with weight loss. Notably, as observed for PAI-1, CRP values decreased with weight loss to levels lower than those in controls. The decreases even below the control values observed are the same as we have observed for many other markers assessed in morbidly obese patients undergoing weight loss by bariatric surgery.¹⁶

EFFECTS OF BMI AND WEIGHT LOSS ON COAGULATION AND INFLAMMATION MARKERS

The **Figure** shows the changes in the different measurements as a function of BMI before and after weight loss. The upper left panel shows the mean BMIs in the 4 subgroups studied and the means in these groups 1 year after surgery. Three conclusions can be drawn about the measurements shown in the Figure. First, measurements in obese patients tended to be higher in those with higher BMIs in a more or less pronounced way, except for sCD40L and CRP levels, which tended to be lower in those with BMIs greater than 55. Second, after surgery, the recovery in the different measurements is also a function of BMI, and for some markers, the postoperative slope between BMI groups was almost parallel to the preoperative slope (see ATIII and fibrinogen). Third, for PAI-1 and CRP, the mean control values were significantly lower than those in obese patients for all BMIs, but these measurements not only recovered to normal values after weight loss but fell below them. For fibrinogen and ATIII, control values were lower than those in both obese and postoperative patients for all BMIs, and patients who lost weight actually had higher ATIII activity levels than obese patients, for all BMIs.

The profile of sCD40L is different from those of the other markers measured, for 2 reasons. First, because of dispersion of the data, the difference between obese and postoperative patients was not significant. Second, when weight loss was observed, a very steep increase in sCD40L levels was noted between BMI groups (from <45 to 60), with almost all values significantly higher than control values. The small sample size of some of our subgroups (eg, patients with BMIs >55) could limit our ability to obtain conclusive results about sCD40L in this group.

Table 1. Characteristics of Obese Patients Before and After Bariatric Surgery

Characteristic	Mean (SEM) Values	
	Obese Patients	Patients 12 mo After Surgery
BMI	48.8 (0.9)	30.9 (0.9) ^a
Waist circumference, cm	136.1 (2.3)	98.5 (2.9) ^a
Hip circumference, cm	145.0 (2.3)	117.8 (3.0) ^a
HOMA-IR	7.7 (1.1)	1.9 (0.3) ^a
Platelet count, $\times 10^9/\mu\text{L}$ plasma	286 (12)	268 (12) ^b
Platelet volume, %	8.98 (0.17)	9.29 (0.20) ^b
Calcium, mg/dL	8.9 (0.1)	9.2 (0.1) ^c
Iron, $\mu\text{g}/\text{dL}$	67.1 (4.9)	86.4 (5.7) ^c

Abbreviations: BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); HOMA-IR, homeostasis model assessment–insulin resistant; SEM, standard error of mean.

SI conversion factors: To convert calcium to millimoles per liter, multiply by 0.25; iron to micromoles per liter, multiply by 0.179; and platelet count to $\times 10^9/\text{L}$, multiply by 1.0.

^a $P < .001$ for difference between patient groups (2-tailed paired t test).

^bDifference not significant.

^c $P < .05$.

COMMENT

The novelty of our study is that all measured markers in obese patients, except for sCD40L, increase with BMI. The results of our study show for the first time that sCD40L levels did not differ significantly between obese men and women for any BMI. However, we observed that sCD40L levels in men can increase or decrease with surgery in the BMI subgroups studied, but in women they tended to increase with surgery in all BMI subgroups (except 50-55). When we considered men overall, combining all BMI groups, we noted a significant decrease in sCD40L levels with surgery in men.

We found sCD40L values ranging from 2.2 pg/mL in the control group to 370 pg/mL in obese patients with BMIs lower than 45 and between 37 and 1353 pg/mL in those with BMIs 50 to 55. Unek et al¹⁷ reported differences in sCD40L levels between patients with and those without metabolic syndrome (with BMIs of 32 and 25, respectively) but not among patients with normal glucose tolerance, prediabetes, or diabetes.

The values reported for sCD40L in the literature are different from our findings. Reported serum concentrations of sCD40L have ranged from 1 to 7 ng/mL in obese patients with BMIs of 45,¹⁸ from 8.06 to 9.51 ng/mL in obese patients with BMIs ranging from lower than 25 to 35 or higher,¹⁹ and from 1.6 to 2.8 ng/mL after 1 year of weight loss.²⁰ Some authors even observed no differences in serum levels between obese and normal-weight patients (8.63 vs 8.06 ng/mL, respectively).¹⁹ The discrepancies between studies may reflect differences in patient BMIs, differences in the kits used in assessments, and/or the differences between serum and plasma levels. We used EDTA-treated platelet-poor plasma. The CD40L is present in platelet granules and is released on platelet activation. Therefore, platelet-free plasma should be used for measuring circulating levels of CD40L.

Table 2. Sex and Surgery Effects in Markers of Coagulation and Inflammation

Marker	Mean (SEM) Values						P Values for 2-Way ANOVA ^a			
	All		Men		Women		Sex Effect	Surgery Effect	Sex-Surgery Effect	
	Controls	Obese	Obese	12 mo After Surgery	Obese	12 mo After Surgery				
sCD40L, pg/mL	2.2 (1.0)	276.1 (53.1) ^b	222.3 (45.0) ^b	246.5 (70.4) ^b	82.2 (23.2) ^{b,c}	285.9 (67.5) ^b	287.0 (56.9) ^{b,c}	.08	.24	.24
PAI-1, ng/mL	75.9 (10.4)	154.6 (16.1) ^b	34.7 (5.5) ^{b,d}	174.0 (26.9) ^b	46.3 (14.6) ^e	146.9 (22.2)	31.6 (6.2) ^{b,d}	.38	<.001	.73
ATIII activity, %	104.2 (4.0)	120.0 (2.0) ^b	129.7 (1.4) ^{b,d}	116.8 (3.9)	128.8 (2.2) ^{b,c}	120.8 (2.6) ^f	129.8 (2.0) ^{b,d}	.53	<.001	.83
Fibrinogen, mg/mL	2.8 (0.3)	4.0 (0.1) ^b	3.4 (0.1) ^{e,g}	4.4 (0.2) ^f	3.4 (0.3) ^e	3.9 (0.1) ^b	3.5 (0.2) ^c	.27	<.001	.27
CRP, µg/mL	6.9 (0.9)	21.3 (1.8) ^b	4.1 (0.6) ^{d,f}	29.3 (3.1) ^b	5.6 (1.4) ^d	21.3 (1.9) ^b	4.6 (0.8) ^{d,h}	.65	<.001	.65

Abbreviations: ANOVA, analysis of variance; ATIII, antithrombin III; CRP, C-reactive protein; PAI-1, plasminogen activator inhibitor-1; sCD40L, soluble CD40 ligand; SEM, standard error of mean.

SI conversion factors: To convert C-reactive protein to nanomoles per liter, multiply by 0.524; fibrinogen to micromoles per liter, multiply by 0.0294.

^aTwo-way ANOVA was used to study the interaction between sex and the effect of surgery (weight loss).

^b $P < .001$ (2-tailed unpaired *t* test comparing control and obese groups).

^c $P < .05$ (2-tailed paired *t* test comparing obese patients before and 12 mo after surgery).

^d $P < .001$ (2-tailed paired *t* test comparing obese patients before and 12 mo after surgery).

^e $P < .01$ (2-tailed paired *t* test comparing obese patients before and 12 mo after surgery).

^f $P < .01$ (2-tailed unpaired *t* test comparing control and obese groups).

^g $P < .05$ (2-tailed unpaired *t* test comparing control and obese groups).

^h $P < .05$ (comparison between male and female patients).

However, not all authors find differences between levels of sCD40L in plasma and those in serum.^{21,22} Moreover, according to the supplier datasheet of the kit used in our study, the expected values in plasma for a healthy person would range from undetectable to 139 pg/mL and those in serum from undetectable to 11 451 pg/mL. In our study, we observed that at any BMI, the obese group had higher sCD40L levels than controls, but we did not observe these values to increase significantly with BMI, in contrast to what other authors have described.¹⁷ One possible reason for this divergence is that we studied patients with BMIs ranging from 35 to 60 and normal-weight controls (BMI <25), whereas other studies included participants with BMIs lower than 25, 25 to 30, or higher than 30.¹⁷ The same distinction applies to the study conducted by Guldiken et al,¹⁹ which included persons with BMIs lower than 25, 30 to 35, or higher than 35. Guldiken et al¹⁹ observed no significant differences in the values of sCD40L between normal-weight and obese patients. The small sample sizes of some of our groups (eg, patients with BMIs >55) and the wide dispersion of data before and after weight loss could limit our ability to obtain conclusive results concerning sCD40L levels.

We have also been unable to find any correlation between sCD40L levels in the obese group and many anthropometric and biochemical markers measured, including body weight, excess weight, and waist and hip circumferences; levels of triacylglycerols, fatty acids, glycerol, cholesterol, CRP, leptin, ghrelin, and lipases; and blood cell counts (eg, monocyte and leukocyte counts; data not shown). This lack of correlation between sCD40L levels and anthropometric markers in obese patients was also observed by Guldiken et al.¹⁹

The only correlations with sCD40L levels that we found in obese patients were plasma iron ($r = 0.65$) and calcium ($r = 0.62$) levels ($P < .01$ for both). The sCD40L is a proinflammatory and prothrombotic ligand, and the latter characteristic could help explain its association with calcium. The extension of the clot formed by platelets is key to the increased cytosolic calcium,^{23,24} although the

prothrombotic mechanism could be offset by the increase in ATIII that we observed. The presence of EDTA (a strong chelator of calcium) as an anticoagulant in blood collection is considered effective in inhibiting the release of sCD40L, but only when added before platelet activation,²⁵ which was not done in our study. We also found a positive correlation ($r = 0.52$; $P < .01$) between PAI-1 and sCD40L levels.

We found no other correlations, neither in obese patients only nor after weight loss, although other authors have found that platelet counts correlate well with sCD40L levels in obese patients.²² The increase in CRP, according to the degree of obesity, has been observed by other authors,^{17,19} although in a much lower range than in our study. Thus, to our knowledge, this work demonstrates for the first time not only that CRP continues to increase up to a BMI of 60 but also that when this marker decreases as a result of weight loss, it decreases in proportion to the initial BMI, to values below those of patients with normal weight. Hanusch-Enserer et al²⁰ have studied some measurements that they consider nonconventional markers of atherosclerosis (eg, PAI-1, CRP, and sCD40L levels) in patients with BMIs higher than 40, before and 1 year after adjustable laparoscopic gastric banding. Their results for PAI-1 and CRP were much lower than what we observed (Table 2) both before surgery (data shown as means [SDs]; CRP, 5.7 [0.6] mg/dL; PAI-1, 44.1 [15.3] ng/mL) and after surgery (CRP, 5.5 [0.4] mg/dL; PAI-1, 34.9 [10.4] ng/mL). However, the type of surgery performed in our study, gastric bypass, was also different. For sCD40L, these authors²⁰ found values of 1.6 (1.5) and 2.2 (1.9) ng/mL before and after surgery (we observed 0.28 [0.05] and 0.22 [0.05] ng/mL, respectively). Hanusch-Enserer et al²⁰ proposed, based on their data, that only PAI-1 is a good marker of inflammatory and prothrombotic response in extreme obesity.

Despite the small sample size ($n = 4$) of 1 BMI subgroup in our study, we believe that we can begin to draw some conclusions from our study results. We propose that, given the marked increase in both PAI-1 and CRP as BMIs

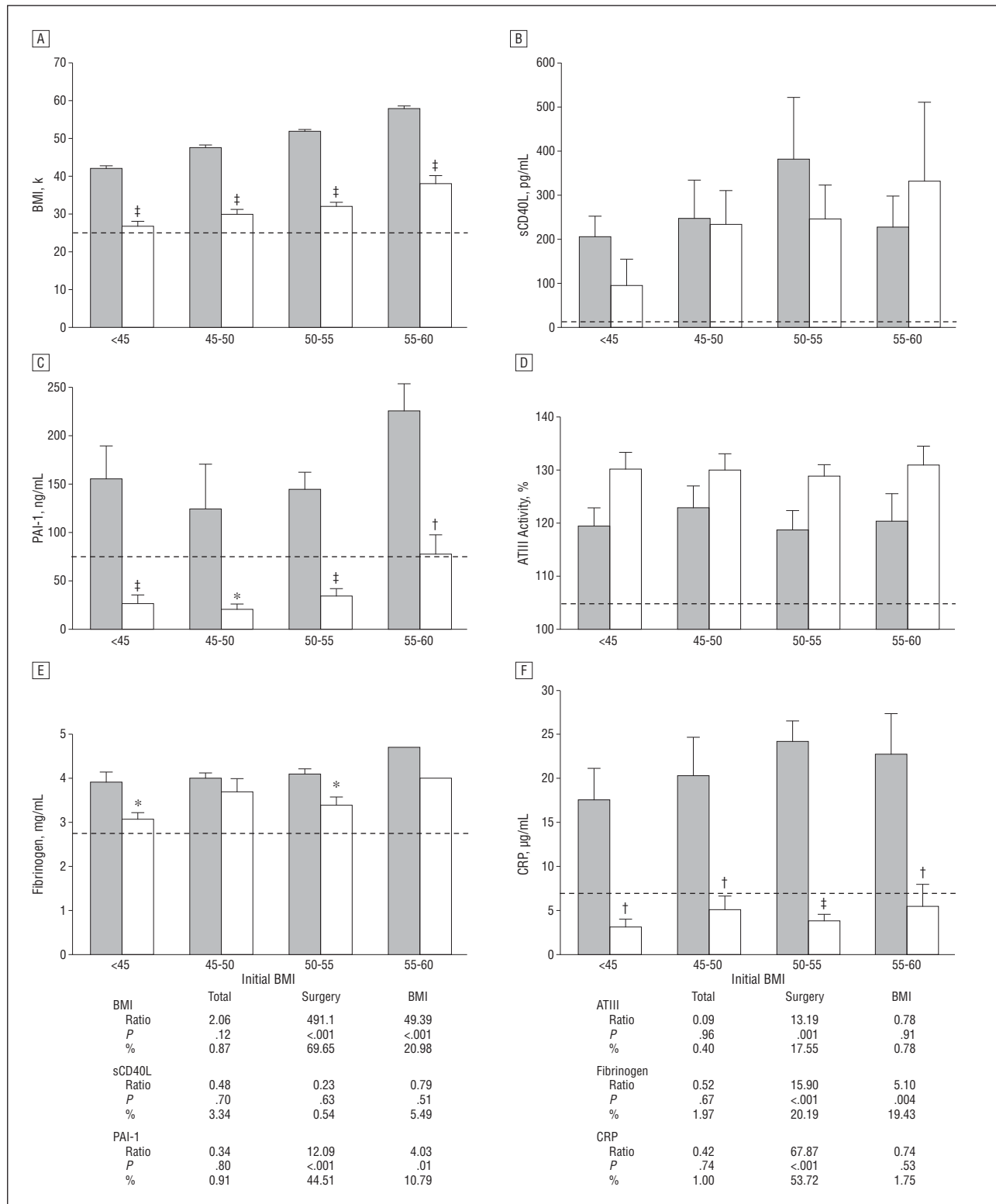


Figure. Body mass index (BMI) and plasma levels of soluble CD40 ligand (sCD40L), plasminogen activator inhibitor 1 (PAI-1), antithrombin III (ATIII), fibrinogen, and C-reactive protein (CRP) in obese patients before gastric bypass (black bars) and the same patients 12 months after surgery (white bars). Values are expressed as means and standard errors of the mean for different BMI ranges; dotted lines indicate mean values in the control (normal-weight) group. Statistical significance was determined with 2-way analysis of variance for the interaction between BMI and surgery ("Total" in the tables); individual comparisons were made with the Bonferroni multiple-comparison test. * $P < .05$; † $P < .01$; and ‡ $P < .001$.

increased, and its evident decrease with weight loss in terms of BMI, those could also be good markers of both risk of cardiovascular and inflammatory disease and/or

prothrombotic states. Soluble CD40L would be an added value in the demonstration of improvement with weight loss.

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Author Affiliations: Surgery Unit, Hospital Universitari Arnau de Vilanova, Lleida, Spain (Dr Baena-Fustegueras); Systemic Autoimmune Diseases Unit (Dr Balada), Biochemistry Department (Ms Ferrer and Dr Catalán), CIBER de Diabetes y Enfermedades Metabólicas Asociadas (CIBERDEM), Instituto de Salud Carlos III, Diabetes Research Unit (Dr Lecube), Endocrinology Surgery Unit (Dr Fort), and CIBER de Enfermedades Hepáticas y Digestivas (CIBEREHD), Instituto de Salud Carlos III (Dr Vargas), Institut de Recerca Vall D'Hebron, Universitat Autònoma De Barcelona; Hospital Universitari Mútua Terrassa, Barcelona (Dr Rivero); Biochemistry and Molecular Biology Department, Biology Faculty (Drs Pardina and Peinado-Onsurbe) and Centres Científics i Tecnològics, Unitat de Tècniques Separatives, Universitat de Barcelona, Barcelona (Dr Casals), Spain.

Correspondence: Julia Peinado-Onsurbe, PhD, Department of Bioquímica y Biología Molecular, Facultat de Biologia, Universitat de Barcelona, Diagonal 645, 08028 Barcelona, Spain (jpeinado@ub.edu).

Author Contributions: Drs Baena-Fustegueras and Peinado-Onsurbe had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. *Study concept and design:* Baena-Fustegueras, Pardina, Balada, Ferrer, Catalán, Rivero, Lecube, Fort, Vargas, and Peinado-Onsurbe. *Acquisition of data:* Pardina, Balada, Ferrer, and Catalán. *Analysis and interpretation of data:* Baena-Fustegueras, Pardina, Balada, Ferrer, Catalán, Rivero, Lecube, Fort, Vargas, and Peinado-Onsurbe. *Drafting of the manuscript:* Baena-Fustegueras, Pardina, and Peinado-Onsurbe. *Critical revision of the manuscript for important intellectual content:* Baena-Fustegueras, Pardina, Balada, Ferrer, Catalán, Casals, Rivero, Lecube, Fort, Vargas, and Peinado-Onsurbe. *Statistical analysis:* Baena-Fustegueras, Pardina, and Peinado-Onsurbe. *Administrative, technical, or material support:* Baena-Fustegueras, Pardina, and Peinado-Onsurbe. *Study supervision:* Baena-Fustegueras, Pardina, Balada, Ferrer, Catalán, Casals, Rivero, Lecube, Fort, Vargas, and Peinado-Onsurbe.

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