Effect of bariatric surgery on microvascular dysfunction associated to metabolic syndrome: a 12-month prospective study

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Abstract

Objective: To prospectively evaluate the effect of weight loss after bariatric surgery on microvascular function in morbidly obese patients with and without metabolic syndrome (MetS).

Methods: A cohort of morbidly obese patients with and without MetS was studied before surgery and after 12 months of surgery. Healthy lean controls were also examined. Microvascular function was assessed by postocclusive reactive hyperemia (PORH) at forearm skin evaluated by laser Doppler flowmetry (LDF). Regression analysis was performed to assess the contribution of different clinical, metabolic and biochemical parameters to microvascular function

Results: Before surgery, 62 obese patients, 39 with MetS and 23 without MetS, and 30 lean control subjects were analyzed. The absolute area under the hyperaemic curve (AUC_H) of PORH was significantly decreased in obese patients compared to lean control subjects. One year after surgery, AUC_H significantly increased in patients free of MetS, including patients that had MetS before surgery. In contrast, AUC_H did not significantly change in patients in whom MetS persisted after surgery. Stepwise multivariate regression analysis showed that only changes in HDL cholesterol and oxLDL independently predicted improvement of AUC_H after surgery. These two variables together accounted for 37.7% of the variability of change in AUC_H after surgery.

Conclusions: Bariatric surgery could significantly improve microvascular dysfunction in obese patients, but only in patients free of MetS after surgery. Improvement of microvascular dysfunction is strictly associated to postoperative increase in HDL-C levels and decrease in OxLDL levels.

Keywords: bariatric surgery, microvascular dysfunction, metabolic syndrome, HDL-cholesterol, oxidized LDL, laser Doppler flowmetry

Introduction

Obesity is characterized by impaired microvascular function that may contribute to increased risk of cardiovascular disease [1]. Clinical and experimental evidence suggest that this microvascular dysfunction may also contribute to obesity-associated hypertension and insulin resistance, which are major cardiovascular risk factors[2, 3]. Several methods are used to assess microvascular function in clinical research[4]. Postocclusive reactive hyperemia (PORH) at forearm skin evaluated by laser Doppler flowmetry (LDF) has been widely used to assess microvascular function due to its non-invasive nature. PORH is the sudden rise in skin blood flow after release of a brief arterial occlusion and provides an overall measurement of microvascular function[5]. However, concerns about the reproducibility of this method have been recently raised[5]. In particular, reproducibility studies of LDF in obese patients are scarce.

Bariatric surgery has emerged as an effective treatment for morbid obesity based on both its efficacy[6] and beneficial effects on obesity-related comorbidities and total mortality[7-9]. It has been previously shown that bariatric surgery could reverse microvascular dysfunction in obese patients although the determinants that mediate this improvement in microvascular function have not been identified[10-14]. Bariatric surgery-induced weight loss also ameliorates the metabolic syndrome (MetS), a group of clinical manifestations that includes obesity, hypertension, insulin resistance, and dyslipidemia[15]. Interestingly, the association between surgically induced improvement in microvascular function and metabolic syndrome has not been previously investigated.

The purpose of this study was to investigate the effect of surgically induced weight loss on microvascular function in morbidly obese patients with and without MetS. More specifically, we wanted to determine whether changes in microvascular function after bariatric surgery are associated to resolution of MetS. To this end, we prospectively evaluated microvascular function by LDF in morbidly obese patients with and without MetS before and 12 months after bariatric surgery.

We also assessed the contribution of different clinical, metabolic and biochemical parameters to surgically induced improvement in microvascular function.

Materials and Methods

Study design and subjects

Obese subjects were recruited from the waiting list for bariatric surgery of the Surgery Unit at Hospital Universitario Virgen del Rocío from November 2009 to March 2011. All obese patients were required to meet NIH guidelines for eligibility for bariatric surgery: BMI ≥40 kg/m^2 or ≥35 kg/m^2 with comorbidities (i.e. diabetes, hypertension, dilated cardiomyopathy or sleep apnea) [16]. The inclusion criteria were male and female patients aged 16–65 years, and agreement to participate in the study by providing a signed consent form. The patients with arterial hypertension, diabetes, cardiomyopathy and sleep apnea were under medical treatment for these obesity complications at the time of the evaluation. Exclusion criteria included acute or chronic inflammatory disease, malignant disease, asthma or any history of alcohol or drug abuse. The experimental protocol was approved by the Ethical Committee of the Hospital Universitario Virgen del Rocío. All participants provided written informed consent to participate in the study. Additional written informed consent was obtained after the surgical procedure.

Obese patients were grouped as patients with MetS or without MetS based on the definition of MetS proposed by the Third Report of the National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults Panel III [17]. Patients fulfilling three or more of the following criteria were considered as having MetS: 1) central obesity (waist circumference >102 cm in men or >88 cm in women); 2) high blood pressure of 130/85 mm Hg or greater or use of antihypertensive therapy; 3) high fasting glucose (≥110 mg/dL); 4) hypertriglyceridemia (≥150 mg/dL), and 5) low high-density lipoprotein cholesterol (HDLc) (<40 mg/dL for males or <50 mg/dL for females). Healthy lean subjects were also recruited as a control group.

Each subject made a visit at baseline and 12 months after the bariatric surgery. For the 12-month follow up study, patients in the group with MetS before surgery were further subdivided into two subgroups: patients in whom MetS resolved after surgery and patients in whom MetS persisted after surgery.

Clinical and biochemical measurements

Clinical and biochemical measurements were performed before surgery (two weeks earlier) and 12 months after surgery. Every measurement was performed after an overnight fast of 10 h. The systolic and diastolic blood pressure values were the mean of two measurements with subjects in sitting position. Measures of weight, height, and waist and hip circumferences were also obtained. Blood samples were drawn from an antecubital vein. Plasma glucose, total serum cholesterol, high-density lipoprotein and triglycerides were measured using a Cobas® C chemistry analyzer (Roche Diagnostics, Mannheim, Germany). The Friedewald equation was used to calculate LDL cholesterol (LDLc) from total serum cholesterol, HDL cholesterol (HDLc), and triglycerides [18]. Plasma insulin was measured by electrical chemiluminescence immunoassay using an ElecsysE170 (Roche Diagnostics, Mannheim, Germany. Haemoglobin A1c (HbA_{1c}) was measured by high-pressure liquid chromatography using a Variant II® analyzer (BioRad Laboratories, Hercules, USA) The index of insulin resistance (HOMA) was calculated using the formula: glucose (mmol/L) X insulin (μU/mL)/22.5. Values greater or equal to 3 were considered indicators of insulin resistance. Plasminogen activator inhibitor-1 (PAI-1) concentrations were determined by enzyme-linked immunosorbent assay (American Diagnostica Inc, Stamford, USA). Ultrasensitive C-reactive protein (CRP) was measured with the CardioPhase® hsCRPkit (Dade Behring, Marburg, Germany), being the intra and interassay CVs 2.8 and 4.6%, respectively. Oxidized low-density lipoprotein (oxLDL) was measured with the ELISA kit (Immunodiagnostic Systems, Boldon, UK.). The intra e inter-assay CVs were 3.9 and 9%, respectively.

Post-occlusive forearm skin reactive hyperaemia (PORH) measurements

Studies were performed in the morning, in a quiet, temperature-controlled room (22–24 °C). Subjects were asked to avoid smoking and caffeine- and alcohol-containing drinks for 24 hours, and from performing vigorous exercises for at least 12 hours before the test. Measurements were taken with subjects in a supine position. On the preoperative study, LDF test was performed two weeks before the surgery.

Changes in cutaneous blood flow (flux) was measured by a commercial single-point laser Doppler flowmetry device (Periflux 5000; bandwidth of 15 kHz, Perimed AB, Järfälla, Sweden) with a thermostatic laser Doppler probe (Probe 481-1, Perimed AB, Järfälla, Sweden). The probe has a fiber separation of 0.25 mm and collects perfusion data at a depth of about 0.5-1 mm. Blood flow data were recorded continuously at a sample rate of 40 recordings per second. Data from the laser Doppler perfusion monitor were analyzed using PeriSoft for Windows, version 2.5.5 (Perimed AB, Järfälla, Sweden). Data files were processed for conversion from mV to PU (perfusion units) by division with the gain factor of the instrument (10 mV/PU). The laser Doppler probe was placed on the volar surface of the right forearm, 10 cm proximal to the wrist. This position was marked so that exactly the same site was used in all measurements. After a baseline measurement of 3 minutes, the brachial artery was occluded using a pressure cuff placed around the right upper arm that was inflated to 220 mmHg. Inflating the cuff took less than 5 seconds. This local ischemia was held for 4 minutes, and then deflated. Deflating the cuff was practically instantaneous (< 40 mmHg within 0.2 seconds). The flux recording was continued for at least 5 minutes (until the signal reaches the baseline flux). Five different PORH parameters were analyzed (supplementary Figure 1). The value of skin flux at baseline is defined as the average value of the 3-minute baseline period before occlusion. Maximum response (PORHmax) was defined as the maximum absolute change (PORHpeak) from baseline [19]. The area under the hyperaemic curve (AUC_H) was calculated from the time the cuff was released until the end of the measurement. The area under the occlusion curve (AUC_O) was calculated from the time the occlusion started until the end of the occlusion. To determine the reproducibility of laser-Doppler-derived parameters in the measurement of PORH in obese patients, a study of reproducibility was performed before and after bariatric surgery. Specifically, we investigated within-

subject reproducibility of the PORH parameters, i.e., reproducibility and variability between measurements. These measurements were performed at different times: (0, 15 min and 24 hours after the first measurement). All measurements were performed by the same investigator.

Statistical methods

Results are shown as the mean ±SD or median and interquartile range unless otherwise noted. Between-group differences in normally distributed data were assessed by one-way analysis of variance (ANOVA) followed by the Fisher's multiple comparison tests to identify differences between groups. For non-normally distributed data, comparisons between groups were analyzed by the Kruskal-Wallis test with Dunn's multiple comparison tests. To analyze changes after bariatric surgery, the two-way ANOVA for repeated measures was chosen with post hoc Tukey's comparisons. The contribution of clinical and biochemical parameters to variation in microvascular function was assessed by multivariate regression analysis. Statistically significant predictors were included in the models with a stepwise procedure, after adjusting for age and gender, and antihypertensive and diabetic treatment. Two sided *p* values were assessed for all models. Only variables that had a $p<0.05$ were included in the final model. To determine the reproducibility of LDF, the intraclass correlation coefficients (ICC) were calculated. Values of intraclass correlation coefficient more than 0.80 were considered excellent reproducibility [20, 21]. Studies were also compared pairwise to check the precision of the method, the existence of magnitude-dependent bias and systematic error by using the Bland & Altman plots. Statistical analyses were performed with the SPSS statistical package (version 17.0; SPSS, Chicago, IL). Power calculation indicated that our sample size provided an 80% power to detect differences in the vascular reactivity with an effect size as low as 0.2 (Cohen's f), and 90% power to detect an effect size as low as 0.23, based on two-sided tests at the 0.05 significance level. Power calculations were conducted using GPower3 [22].

Results

Before analyzing the impact of the MetS on microvascular function, we wanted to determine the reproducibility of the different LDF parameters in obese patients. ICC analysis showed that all LDF measurements were above 0.80 (supplementary Table 1). Bland-Altman plots displayed no apparent trend or evidence of systematic bias (supplementary Figures 2 and 3). Thus, all LDF parameters showed a high repeatability with good high within-observer reproducibility in our study. Between-group comparisons in LDF measurements are shown in Table 1. AUC_H was significantly decreased in both groups of obese patients compared to lean control subjects. There were no significant differences in this variable between the two groups of obese patients (p = 0.958).

A total of 62 patients (53 women; age range 19–65 years) completed the 12-month follow-up assessment. Clinical and metabolic characteristics of the obese patients and 30 lean control subjects are described in Table 2. All groups were matched for age and gender. As expected, there was a high prevalence of diabetes and hypertension among obese patients with MetS. Systolic blood pressure, fasting glucose, HbA_{1c}, total cholesterol, LDLc and triglycerides concentrations were higher in obese patients with MetS compared to both obese subjects without MetS and lean subjects. Diastolic blood pressure, heart rate, and fasting insulin were higher in the two obese groups. Furthermore, PAI-1, CRP and oxLDL levels were higher in obese patients when compared to lean control subjects.

One-year follow-up data for all patients are shown in Table 3. Forty-five and 17 patients underwent laparoscopic sleeve gastrectomy and Roux-en-Y gastric bypass, respectively. No differences in weight loss between the different types of bariatric surgery were observed (p = 0.223). The disparity between male and female patients in the current study (85.5% women) is in agreement with studies reporting that women seek bariatric surgery more often than men [23]. However, no significant differences were found among the 3 study groups in terms of gender and type of surgery (p = 0.798 and p = 0.963, respectively). Resolution of MetS was observed in 27 patients. Bariatric surgery reduced significantly anthropometric values in all patients. However, patients with MetS after surgery displayed higher BMI than patients without MetS. Higher waist and hip circumference was observed

in patients with MetS after surgery compared to patients in whom MetS was resolved after surgery. Bariatric surgery resulted in a decrease in DBP and heart rate in patients in whom MetS was resolved after surgery. All patients reduced fasting insulin, HOMA-IR, Hb_{A1c}, PAI-1, and CRP levels after surgery. A significant improvement in fasting glucose, total cholesterol, LDLc and HDLc, triglycerides and oxLDL was observed in patients free of MetS but not in patients with MetS after surgery. Comparison between groups showed elevated fasting glucose, fasting insulin, HOMA-IR, Hb_{A1c}, triglycerides, CRP and oxLDL levels in the group of patients in whom MetS persisted after surgery compared to the other groups of obese patients. Low HDL cholesterol was also observed on these patients compared to the other groups.

Bariatric surgery resulted in a significant increase in AUC_H in patients free of MetS, including patients that had MetS before surgery ($p < 0.05$) (Figure 1). In contrast, AUC_H did not significantly change in patients in whom MetS was not resolved after surgery ($p = 0.72$). To identify determinants of microvascular function changes after surgery, correlation analysis were performed between all clinical and biochemical variables and AUC_H. Increased AUC_H after bariatric surgery was significantly associated with an increase in HDLc (Pearson's R = 0.53, $p < 0.001$) and a decrease in oxLDL (R = -0.54, $p < 0.001$), fasting glucose (R = -0.27, $p = 0.04$) and HbA_{1c} (R = -0.29, $p = 0.03$). When these biochemical variables were entered into a stepwise multivariate regression analysis, only changes in HDLc and oxLDL concentrations independently predicted improvement of AUC_H after surgery (Table 4). These two variables together accounted for 37.7% of the variability of change in AUC_H after surgery. Interestingly, HDLc levels correlated significantly with oxLDL (R = -0.30, $p = 0.03$).

Discussion

Our prospective study reveals an association between MetS and surgically induced improvement of microvascular dysfunction in obese patients. This improvement in microvascular dysfunction is independently associated with decreased oxidized LDL and increased HDL cholesterol.

To assess microvascular function, we utilized PORH at forearm skin evaluated by LDF, a method widely used in vascular research [5]. PORH has been shown to be fairly reproducible in lean subjects [24-26]. Our ICC analysis extends these results demonstrating that all LDF parameters of our study are highly reproducible both intraday (within-day) and interday (between-day) in obese patients. Several different parameters can be obtained when performing PORH [5]. AUC_H is a commonly used parameter that simultaneously measures velocity, intensity and duration of the hyperemia response [16]. Several studies have demonstrated that AUC_H is a reliable indicator of microvascular dysfunction in patients at risk of cardiovascular disease [27-30]. Our LDF studies performed before surgery revealed that AUC_H was significantly decreased in obese patients compared to lean control subjects. These results add to the growing body of evidence that microvascular function is impaired in obese patients [1, 2].

Surgically induced weight loss resulted in a significant improvement in microvascular function in obese patients without MetS, including patients that had MetS before surgery. These results are in agreement with previous studies showing that microvascular dysfunction could be reversed in obese patients after successful bariatric surgery [10-14]. However, our results reveal that obese patients with MetS after surgery, despite significant weight loss, still exhibit microvascular dysfunction. These findings suggest that MetS is a strong determinant of improvement of microvascular function associated with surgically induced weight loss. Stepwise multiple linear regression analysis revealed that low HDLc levels is the component of MetS that best predicts lack of improvement in microvascular dysfunction in obese patients.

In addition to HDLc, our regression analysis identified oxLDL as an independent predictor of improvement of microvascular function in obese patients. An increase in HDLc levels and a decrease in oxLDL levels were independently associated with improvement of microvascular function after surgery. The relationship between HDLc, oxLDL, and AUC_H can, at least in part, explain the lack of improvement in microvascular function in patients in whom MetS persisted

after surgery given that HDLc and oxLDL levels did not significantly change in this group after surgery. Although HDLc and oxLDL are known to play an important role in endothelial function, [31, 32] the contribution of these factors to obesity-related microvascular dysfunction has been less explored. Our results indicate that HDLc and oxLDL are good predictors of improvement in microvascular function in obese patients after surgery.

Our findings that surgically induced weight loss leads to increased HDLc levels and decreased oxLDL levels are in agreement with previous studies [32, 33]. Our study further extends these findings showing that HDLc levels correlate negatively with oxLDL levels in patients after bariatric surgery. These results provide a potential mechanism by which surgically induced weight loss might improve microvascular dysfunction in obesity. Experimental data obtained in human subjects [34] and in animal models [35] have demonstrated that HDL can counteract the inhibitory effect of oxLDL on vascular reactivity. Thus, it is tempting to speculate that bariatric surgery might induce an increase in HDLc levels leading to a reduction in oxidation of LDL and, in turn, an improvement of microvascular function. Why the patients in whom MetS persisted after surgery did not show an improvement in microvascular function remains to be determined. It is important to note that this group of patients, despite significant weight loss after surgery, still remains morbidly obese. Although thi might suggest that the lack of microvascular improvement in this group of patients is due to insufficient weight loss, no independent association was found between the degree of surgically induced weight loss and microvascular function in the whole set of obese patients. Nevertheless, an indirect effect of weight loss in microvascular function through changes in HDLc and oxLDL levels cannot be ruled out.

The main limitation of our study is that we cannot conclude whether the surgically induced improvement in microvascular function in obese patients is endothelium-dependent. The mediators contributing to PORH include endothelial-dependent vasodilation, myogenic responses and sensory nerves. Other microvascular reactivity tests such as iontophoresis of vasodilators (e.g.

acetylcholine) are used as specific tests of endothelium-dependent function [4]. However, the complexity and low reproducibility of these tests poses major limitations for its wide use in clinical research.

In summary, the present study shows that bariatric surgery could significantly improve microvascular dysfunction in obese patients, but only in those patients free of MetS after surgery. This improvement is strictly associated to postoperative increase in HDLc levels and decrease in OxLDL levels. Our results suggest that HDLc and oxLDL are good markers of improvement of microvascular function associated with surgically induced weight loss.

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Supplementary information is available at International Journal of Obesity's website

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FIGURE LEGEND

Figure 1. AUC_H measurements in obese patients before and 1 year after bariatric surgery. Patients were classified in three groups: patients without MetS before surgery (MetS-/MetS-), patients in whom MetS had been resolved after bariatric surgery (MetS+/MetS-), and patients in whom MetS persisted after bariatric surgery (MetS+/MetS-). Data are presented as mean and SD. * $p<0.05$ ** $p<0.001$ (Tukey's test; within-subject comparison).

Table 1. Laser-Doppler results related to post-occlusive reactive hyperemia in obese patients before surgery and control lean subjects. Data are expressed as mean ±SD.

	Ob/MetS- (n=23)	**Ob/MetS+** (n=39)	**Control subjects** (n=30)	***p* value**
PORHpeak	35.9 ±16	36.5 ±16	42.5 ±13	0.214
PORHmax	29.1 ±15	29.1 ±15	35.0 ±12	0.184
AUC_O	1002.4 ±436	990.2 ±510	1124.8 ±462	0.486
AUC_H	734.2 ±441*	767.4 ±448*	1041.5 ±455	**0.022**

Ob/MetS-, obese patients without metabolic syndrome; Ob/MetS+, obese patients with metabolic syndrome.

Post-hoc comparison vs control subjects * $p<0.05$

555 **Table 2.** Clinical and metabolic characteristics of obese patients before surgery
556 and control subjects.
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	Ob/MetS-	**Ob/MetS+**	**Control subjects**	***p* value**
Number	23	39	30	
Gender (F/M)	19/4	34/5	22/8	0.336
Age (yr)	40 ±9	42 ±10	37 ±11	0.109
BMI [mass (kg)/[height (m)]^2]	49.4 ±5***	50.7 ±5***	24.8 ±5	**<0.0001**
Waist circumference (cm)	134.4 ±14***	133.2 ±13***	80.4 ±11	**<0.0001**
Hip circumference (cm)	149.2 ±12***	147.5 ±11***	101.8 ±8	**<0.0001**
SBP (mm Hg)	128 ±15**	137 ±17***†	116 ±8	**<0.0001**
DBP (mm Hg)	76 ±11***	79 ±11***	65 ±6	**<0.0001**
Heart rate	82 ±8***	81 ±8***	66 ±6	**<0.0001**
Diabetes mellitus (%)	0	33†††	0	**<0.0001**
Hypoglycemic use (%)	0	23†††	0	**<0.0001**
Arterial hypertension (%)	32	66†††	0	**<0.0001**
ACEI or ARB use (%)	18	38††	0	**0.002**
Fasting Glucose	87.2 ±8.4	104.2	82.3 ±10.4	**<0.0001**

(mg/dl)		±20.7***†††		
Fasting Insulin (µU/ml)	20.4 ±9.1***	25.4 ±15.2***	7.4 ±4.5	**<0.0001**
HOMA-IR (mg/dl)	4.7 ±2.1	6.8 ±17.1**	1.6 ±1.0	**0.011**
HbA_{1c} (%)	5.8 ±0.4*	6.5 ±1.1***††	5.23 ±0.3	**<0.0001**
Tchol (mg/dl)	179.4 ±36.7	202.8 ±41.3**†	173.3 ±29.1	**0.003**
LDLc (mg/dl)	116.8 ±18.5	139.0 ±35.8***†	103.8 ±25.4	**<0.0001**
HDLc (mg/dl)	50.6 ±13.2	44.8 ±10.7***	55.6 ±10.07	**<0.0006**
Triglycerides (mg/dl)	98.7 ±35.6	134.0 ±60.8***†	68.9 ±23.4	**<0.0001**
PAI-1 (ng/ml)	82.8 (27.0, 132.0)**	61.8 (39.1, 111.0)**	34.6 (19.1, 50.1)	**0.009**
CRP (ng/l)	9.2 (5.2, 14,1)*	8.9 (5.9, 13.6)*	0.9 (0.3, 2.4)	**0.006**
oxLDL (ng/ml)	187.0 (87.2, 536.7)*	167.0 (67.1, 437.0)**	114.0 (26.0, 203.0)	**0.047**

Mean ±SD., number of subjects (*n*), or median (first, third quartiles).

Ob/MetS-, obese patients without metabolic syndrome; Ob/MetS+, obese patients with metabolic syndrome; FFM, free-fat mass; FM, fat mass; SPB, systolic blood pressure; DBP, diastolic blood pressure; ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; HOMA-IR, homeostasis model assessment-estimated insulin resistance; HbA_{1c}, hemoglobin A_{1c}; Tcho, total cholesterol; HDLc, high-density lipoprotein cholesterol; LDLc, low-density

566 lipoprotein cholesterol; PAI-1, plasminogen activator inhibitor-1; CRP, C-reactive
567 protein; oxLDL, oxidized low-density lipoprotein; n.a., not available.
568 *Post-hoc* comparison vs control subjects * $p<0.05$, ** $p<0.01$, *** $p<0.001$
569 *Post-hoc* comparison vs Ob/MetS- † $p<0.05$, †† $p<0.01$, ††† $p<0.001$
570

571

572 **Table 3.** Clinical and metabolic characteristics of obese patients 12 months after
573 surgery.

574

	MetS-/MetS- (n = 23)	**MetS+/MetS-** (n = 27)	**MetS+/MetS+** (n = 12)
Number	23	27	12
BMI [mass (kg)/[height (m)]^2]	33.9 ±5.5***	32.9 ±7.5 ***	40.2 ±8.2 ***†
Waist circumference (cm)	105.4 ±13.0 ***	99.1 ±13.7 ***	115.1 ±12.8 ***†§
Hip circumference (cm)	121.2 ±14.7 ***	112.8 ±16.8 ***	128.5 ±16.6 ***§
SBP (mm Hg)	119.0 ±13.2	121.3 ±13.8	133.4 ±16.8§
DBP (mm Hg)	69.5 ±8.1	70.7 ±9.6***	74.8 ±11.4
Heart rate	69.8 ±11.1***	71.3 ±10.2*	75.7 ±13.2
Fasting Glucose (mg/dl)	77.1 ±7.4***	80.3 ±8.24***	90.3 ±25.42†
Fasting Insulin (µU/ml)	5.47 ±3.2***	6.21 ±3.2 ***	8.6 ±4.1**†
HOMA-IR (mg/dl)	1.1 ±0.6***	1.3 ±0.8***	1.8 ±0.7**†§
HbA_{1c} (%)	5.2 ±0.3***	5.3 ±0.3***	5.8 ±0.7**†§
Tchol (mg/dl)	175.6 ±31.0	188.1 ±36.6*	192.5 ±28.6
LDLc (mg/dl)	100.6 ±29.0**	114.9 ±37.6*	125.4 ±35.6
HDLc (mg/dl)	62.4 ±9.2**	60.5 ±11.1***	49.4 ±11.2†§
Triglycerides (mg/dl)	64.6 ±14.9***	81.6 ±27.1***	94.3 ±24.1†§
PAI-1 (ng/ml)	27.3 [18.6,	22.8 [15.3, 45.2]*	22.9 [15.4, 82.8]*

	48.4][**]		
CRP (ng/l)	1.5 [0.7, 3.2][***]	1.1 [0.5, 2.9][***]	5.2 [2.6, 8.9][*] [††§§]
oxLDL (ng/ml)	62.4 [40.5, 113.5][***]	64.9 [42.16, 70.0][***]	168.9 [147.2, 235.3][†††§§§]

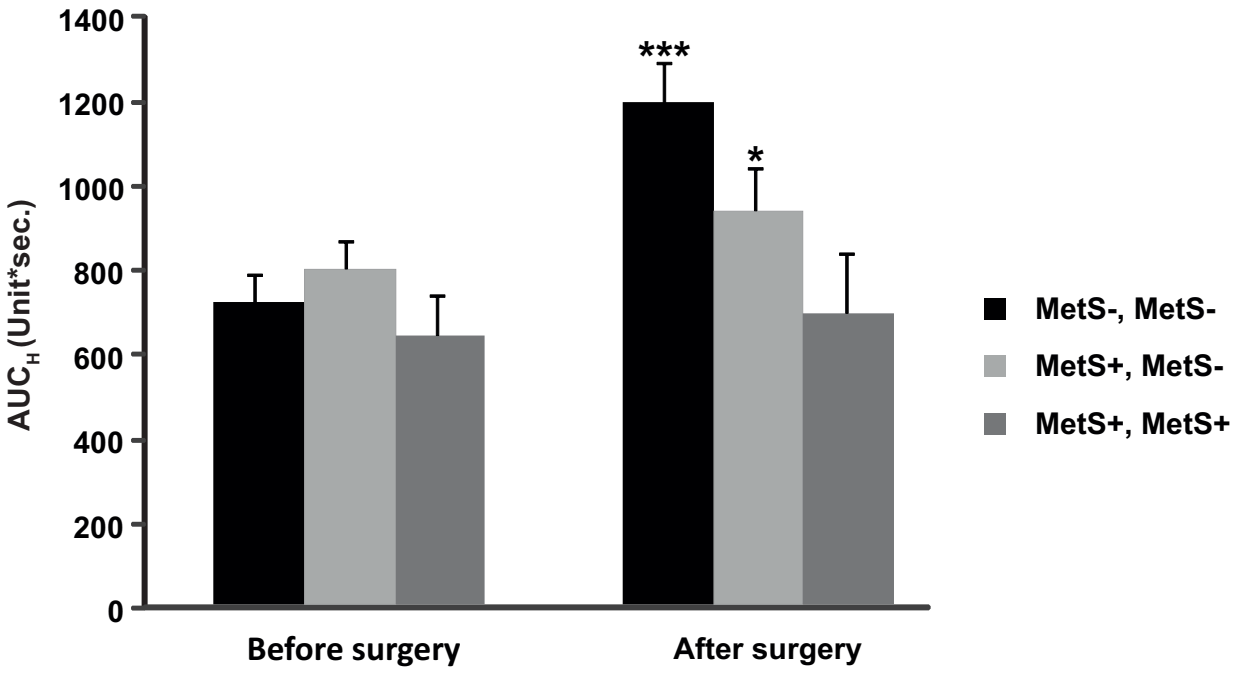
575 MetS-/MetS-: obese patients without metabolic syndrome before and after
576 surgery; MetS+/ MetS-: obese patients in whom metabolic syndrome was resolved
577 after surgery; MetS+/ MetS+: obese patients with metabolic syndrome before and
578 after surgery.
579 Mean ±SD, number of subjects (*n*), or median [first, third quartiles].
580 * $p<0.05$, ** $p<0.01$, *** $p<0.001$ comparing with measures before surgery in the
581 two-way ANOVA.
582 *Post-hoc* comparison vs MetS-/MetS- † $p<0.05$, †† $p<0.01$, ††† $p<0.001$
583 *Post-hoc* comparison vs MetS+/MetS- § $p<0.05$, §§ $p<0.01$, §§§ $p<0.001$
584
585
586

587 **Table 4.** Pearson's correlation coefficients (associated *p*-value) and regression
588 coefficients of a stepwise multiple linear regression analysis in changes in AUC_H
589 (postoperative minus preoperative) as the dependent variable and after forcing
590 age, gender, antihypertensive and diabetic treatment. Independent variables were
591 selected using all variables that correlated significantly with changes AUC_H .
592

Independent variable	Step	Regression coefficient (s.e.)	95% CI	*p*-value	Adjusted R^2 (%)
Change in HDLc (mmol/l)	1	18.48 (5.13)	8.15, 28.81	**<0.001**	27.9
Change in oxLDL (ng/ml)	2	-0.29 (0.08)	-0.12, 0.45	**0.001**	9.8
Change in fasting glucose (mg/dl)		-	-	n.s.	
Change in HbA_{1c} (%)		-	-	n.s.	

593

Figure 1.


1400
1200
1000
800
600
400
200
0
AUC$_H$ (Unit*sec.)
***
*
Before surgery
After surgery
MetS-, MetS-
MetS+, MetS-
MetS+, MetS+