Haemostatic and fibrinolytic changes in obese subjects undergoing bariatric surgery: the effect of different surgical procedures

Roberta Lupoli[1], Marco Milone[2], Alessandro Di Minno[1], Paola Maietta[2], Pasquale Ambrosino[1], Mario Musella[2], Matteo N.D. Di Minno[1,3]

[1]Department of Clinical Medicine and Surgery, [2]Department of Advanced Biomedical Science, Federico II University, Naples; [3]Unit of Cell and Molecular Biology in Cardiovascular Diseases, "Monzino" Cardiology Centre, IRCCS, Milan, Italy

Background. Little is known about effects of different bariatric surgery procedures on haemostatic and fibrinolytic parameters.

Material and methods. Consecutive obese subjects undergoing gastric bypass (GBP) or sleeve gastrectomy (SG) were enrolled. In all patients, levels of haemostatic factors (FII, FVII, FVIII, FIX, FX, vWF, fibrinogen), fibrinolytic variables (PAI-1, t-PA and D-dimer) and natural anticoagulants (AT, protein C and protein S) were evaluated before and 2 months after surgery.

Results. A total of 77 GBP and 79 SG subjects completed the study. At baseline no difference in coagulation parameters was found between the two groups. After both GBP and SG, subjects showed significant changes in haemostatic and fibrinolytic variables and in natural anticoagulant levels. The Δ% changes in FVII, FVIII, FIX, vWF, fibrinogen, D-dimer, protein C and protein S levels were significantly higher in subjects who underwent GBP than in those who underwent SG. Multivariate analysis confirmed that GBP was a predictor of higher Δ% changes in FVII (β=0.268, p=0.010), protein C (β=0.274, p=0.003) and protein S (β=0.297, p<0.001), but not in all the other variables. Following coagulation factor reduction, 31 subjects (25.9% of GBP and 13.9% of SG; p=0.044) showed overt FVII deficiency; protein C deficiency was reported by 34 subjects (32.5% of GBP *vs* 11.4% of SG, p=0.033) and protein S deficiency by 39 (37.6% of GBP *vs* 12.6% of SG, p=0.009). Multivariate analyses showed that GBP was associated with an increased risk of deficiency of FVII (OR: 3.64; 95% CI: 1.73-7.64, p=0.001), protein C (OR: 4.319; 95% CI: 1.33-13.9, p=0.015) and protein S (OR: 5.50; 95% CI: 1.71-17.7, p=0.004).

Discussion. GBP is associated with an increased risk of post-operative deficiency in some vitamin K-dependent coagulation factors. Whereas such deficiency is too weak to cause bleeding, it is significant enough to increase the risk of thrombosis.

Keywords: obesity, haemostasis, fibrinolysis, bariatric surgery, natural anticoagulants.

Introduction

Severe obesity is one of the major health problems in western countries and is associated with several co-morbidities such as an increased incidence of cardiovascular disease[1,2]. In this respect, primary haemostasis (platelet function), fibrinolytic variables (tissue plasminogen activator [t-PA], plasminogen activator inhibitor-1 [PAI-1]) and secondary haemostatic factors (coagulation proteins; natural anticoagulants) are all known to play relevant roles in cardiovascular pathophysiology[3]. Impaired fibrinolysis and/or raised levels of coagulation factors and/or reduced levels of natural anticoagulants (protein C, protein S, antitrombin) have been recognized as major determinants of both arterial and venous thrombosis[4]. Several studies suggest that obesity is characterised by an increased expression of several prothrombotic factors, impaired fibrinolysis and platelet hyper-reactivity[5]. Weight loss has been found to (partially) revert both metabolic and vascular alterations found in obese subjects[1]. Besides nutritional and pharmacological treatments, an increasingly frequent management of severe obesity is bariatric surgery[6]. Based on their effect on food ingestion -reduction in stomach capacity or nutrient absorption- bariatric surgery has been classified as restrictive or malabsorptive, respectively. Although the efficacy of these surgical procedures in weight control and in improving metabolic impairments (e.g. remission of diabetes) has been widely described in several studies[6-8], there is currently insufficient evidence to indicate how to assign a patient to a specific bariatric surgery procedure[9]. Moreover, although it is well known that

the risk of venous thromboembolic events is increased in patients undergoing bariatric surgery, little is known about the effects of different bariatric surgery techniques on haemostatic and fibrinolytic parameters. In this study we prospectively evaluated changes in haemostatic and fibrinolytic variables in obese subjects undergoing gastric bypass (GBP) or sleeve gastrectomy (SG). We also recorded changes in the levels of coagulation factors.

Material and methods

In a 36-month period (January 2009-January 2012), consecutive obese subjects referred to the Federico II University Hospital with an indication (according to the European Association for the Study of Obesity guidelines)[9] for bariatric surgery were evaluated for enrolment in this study. In detail, a patient had to be admitted to undergo bariatric surgery following the failure of a non-surgical weight reduction programme including diet and other interventions (e.g., behavioural modifications, psychotherapy, dietary counselling, or physical training) and in the presence of a body mass index (BMI) >40 or >35 kg/m^2 combined with serious co-existing conditions.

Only patients undergoing GBP or SG were included in the study. Exclusion criteria were indications for surgical procedures other than GBP or SG, known inherited bleeding disease, personal and/or family history of arterial or venous thrombosis, treatment with anticoagulant or antiplatelet drugs, other conditions known to affect haemostasis (liver disease, active inflammatory processes, pregnancy, malignancy, haematological diseases, puerperium, oral contraceptive intake and hormone replacement therapy), a history of chronic infectious disease (including hepatitis B and C) and unstable medical conditions.

Before surgery (T0), information about age, gender, cardiovascular risk factors such as obesity, hypertension, impaired fasting glucose, hypercholesterolaemia, hypertriglyceridaemia and previous and/or current treatments were collected as described previously[10]. In addition, a venous blood sample was drawn from each patient. The sample was taken from the antecubital vein without venous stasis via a 19-gauge scalp-vein needle at 8.30-9.00 a.m. after 12-15 hours of overnight fasting and collected into sterile tubes containing 2 mL sterile 3.8% trisodium citrate. The blood was then centrifuged at 3,000 *g* for 15 minutes and the plasma processed immediately. Fibrinolytic variables (PAI-1, t-PA, PAI-1/t-PA ratio) and haemostatic variables (fibrinogen, D-dimer, coagulation factors II, VII, VIII, IX, X and von Willebrand factor [vWF], protein C, protein S and antithrombin) were determined.

PAI-1 and t-PA antigens (Imulyze™) were measured by enzyme-linked immunosorbent assays using kits from Biopool-Menarini (Florence, Italy)[4]. Fibrinogen activity was evaluated by the Clauss clotting method using a kit from Mascia Brunelli (Milan, Italy). Protein C activity was evaluated by a chromogenic method using a kit from Dade-Behring (Milan, Italy) whereas protein S antigen (Asserachrom®) was measured by enzyme-linked immunosorbent assay using a kit from Diagnostica Stago, Boehringer Mannheim (Milan, Italy)[11]. Levels of coagulation factor II, VII, VIII, IX, and X activity were determined by clotting assays (Dade-Behring, Milan, Italy). vWF antigen was evaluated with a commercially available kit (INNOVANCE® VWF Ac Kit, Siemens Healthcare Diagnostics, Munich, Germany). Antithrombin activity was measured using a commercial kit (Berichrom ATIII, Behringwerke, Marburg, Germany)[12]. Levels of D-Dimer were assessed with the use of the INNOVANCE D-dimer kit (Siemens Healthcare Diagnostics). All laboratory measurements were performed by technicians unaware of the surgical procedure performed in each patient.

All the operations were carried out by a team of expert surgeons, who had performed more than 500 bariatric surgery procedures, and validated criteria were followed for the prevention of surgical site infection and perioperative antiplatelet drug administration[13-15].

All clinical and laboratory evaluations reported above were repeated in all subjects 60 days (±10 days) after surgery (T1). This time point was chosen to identify haemostatic changes occurring in the early post-operative period, while avoiding some potential confounding alterations due to the surgery-induced pro-inflammatory and pro-thrombotic state.

The variability of the laboratory methods was assessed in samples collected twice within a 1-month period, in 260 subjects without any inflammatory, rheumatic or thrombotic disease, recruited in the same period of time from the hospital staff. All changes in laboratory parameters in study patients have been adjusted for the percentage of variability of the method found in the test group.

This study was approved by the Local Ethics Committee of Federico II University.

Statistical analysis

Statistical analyses were performed with the SPSS 16 system (SPSS Inc., Chicago, IL, USA). Continuous data are expressed as means ± standard deviations (SD); categorical variables are expressed as percentages. To compare continuous variables, an independent sample *t*-test was performed. The Wilcoxon test for paired samples was employed as a non-parametric equivalent of the paired-samples *t*-test used for continuous variables. Abdominal obesity, hypertriglyceridaemia, hypercholesterolaemia, hypertension, diabetes and

smoking status were analysed as dichotomous (1/0) categories. The chi-square test was employed to analyse categorical data. When the minimum expected value was <5, Fisher's exact test was used. To adjust for all the other variables, multivariate analyses were performed with Δ% changes in haemostatic and fibrinolytic variables as dependent variables, and with age, gender, type of surgery (GBP *vs* SG), hypercholesterolaemia, hypertriglyceridaemia, diabetes, hypertension, smoking habit, obesity, baseline values of haemostatic and fibrinolytic parameters (FII, FVII, FVIII, FIX, FX, vWF, fibrinogen, D-dimer, antithrombin, protein C, protein S, PAI-1, t-PA) as independent variables. All the results are presented as two-tailed values and are considered statistically significant if the P values are less than 0.05.

In addition, logistic regression analyses, with the same independent variables as reported above, were used the determine the risk of deficiencies of haemostatic parameters.

Sample size evaluation

With the aim of being as conservative as possible, planning a study with a sample size able to detect before-after changes in haemostatic and fibrinolytic variables of more than 5% (±15 as standard deviation within the sampled population), at least 71 subjects for each surgical technique were needed to achieve a greater than 80% power with a α error of 5%.

Results

Of the 213 subjects screened for inclusion in this study, 56 were excluded because of the presence of at least one exclusion criteria. In addition, one subject was excluded from the analysis because he was not re-evaluated at the 2-month follow-up. Thus, a total of 156 obese patients were enrolled in this study, 77 of whom underwent GBP and 79 who underwent SG.

At baseline (T0) GBP and SG subjects were entirely comparable for all major clinical and demographic characteristics (Table I). In addition, all haemostatic and fibrinolytic variables were completely comparable between the two groups of subjects.

An increase in at least one of haemostatic or fibrinolytic parameter above higher normal levels -suggesting a hypercoagulable state- was found in 37 (48.1%) GBP and 40 (50.6%) SG patients (p=0.752).

All surgical procedures were performed without any significant peri-operative complications. No infections or thrombotic complications were recorded.

At the 2-month post-operative follow-up (T1), a 21.3% reduction in BMI was found in the GBP group as compared with a 19.1% reduction in the SG group (p=0.139). In parallel, a reduction in haemostatic and fibrinolytic parameters was recorded in both the GBP and SG groups (Figure 1).

Table I - Clinical and demographic characteristics of the study population before surgery.

Variable	SG N=79	GBP N=77	p
Age (years)	37.9±3.1	38.7±3.9	0.186
Male gender	36 (45.6%)	45 (58.4%)	0.113
BMI	45.3±3.1	44.2±4.7	0.114
PAI-1 antigen	31.7±9.7	33.6±14.5	0.064
t-PA levels	4.8±1.8	5.4±2.0	0.061
Fibrinogen	315.8±48.2	314.1±81.9	0.867
D-dimer	228.8±232.3	211.9±121.3	0.573
FII	113.7±17.9	117.5±19.1	0.213
FVII	124.9±22.2	123.1±22.0	0.613
FVIII	124.6±20.6	123.5±23.4	0.745
FIX	133.9±25.5	132.9±24.2	0.814
FX	97.4±6.8	99.3±7.2	0.094
vWF	136.1±26.5	130.7±22.7	0.173
Protein C	116.7±18.3	118.8±15.4	0.441
Protein S	107.4±18.4	113.1±21.0	0.069
Antithrombin	97.9±6.2	99.7±7.15	0.096
Hypercholesterolaemia	47 (59.5%)	38 (49.4%)	0.206
Hypertriglyceridaemia	27 (34.2%)	25 (32.5)	0.866
Diabetes	39 (49.4)	38 (49.4)	1.000
Hypertension	37 (46.8%)	44 (57.1%)	0.205
Smoking habit	17 (21.5%)	22 (28.6%)	0.357
Obesity	79 (100%)	77 (100%)	1.000

All measurements were performed 1-3 days before surgery.
SG: sleeve gastrectomy; GBP: gastric by-pass.

In detail, Δ% changes in the levels of FVII, FVIII, FIX, vWF, fibrinogen, and D-dimer were significantly greater in the GBP group than in SG group. In addition, GBP patients showed greater changes in protein C and protein S levels, as compared with those undergoing SG. In contrast, no differences were found in changes in antithrombin, FII, FX, PAI-1 and t-PA levels between patients undergoing the two different surgical procedures.

Interestingly, Δ%BMI showed a direct correlation with Δ% changes in fibrinogen (r=0.386, p<0.001), FVIII (r=0.303, p<0.001), vWF (r=0.211, p=0.008) and PAI-1 (r=0.482, p<0.001), but not with any of the other haemostatic parameters. A direct correlation between Δ%PAI-1 and Δ%t-PA was also found (r=0.545, p<0.001).

Multivariate analysis showed that, after adjusting for major clinical and demographic characteristics (including BMI changes), GBP was consistently associated with greater Δ% changes in FVII (β=0.268, p=0.010), protein C (β=0.274, p=0.003) and protein S (β=0.297, p<0.001) levels, but not with any of the other variables.

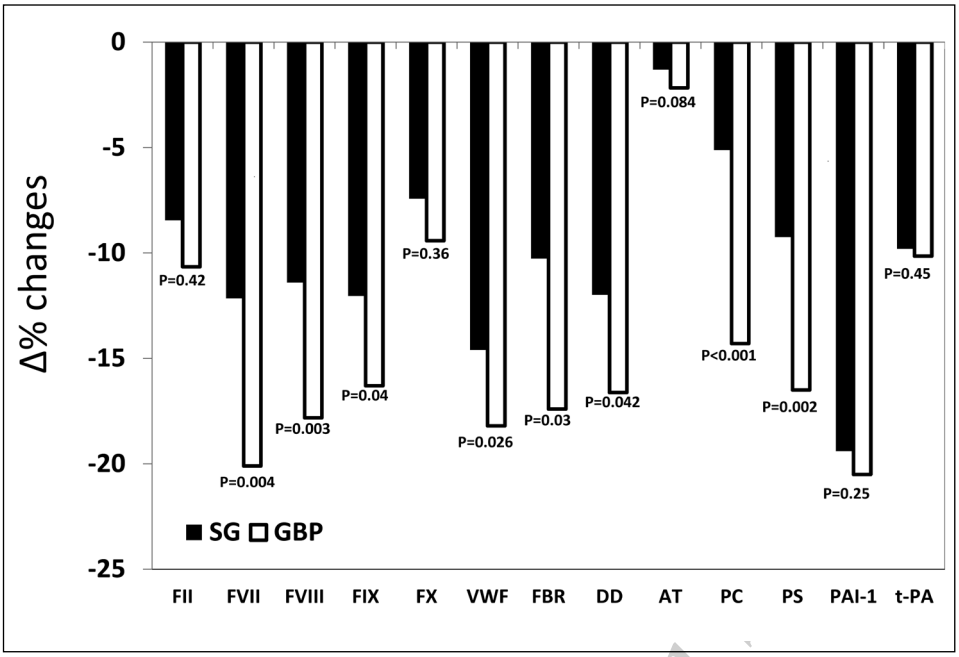

Figure 1 - Δ% changes of haemostatic and fibrinolytic variables 2 months after sleeve gastrectomy (SG) or gastric bypass (GBP).

Interestingly, at the T1 assessment, 31 subjects (25.9% of GBP and 13.9% of SG patients, p=0.044) had FVII levels below lower normal cut-off values (i.e., they had FVII deficiency), whereas no deficiencies of any other clotting factor were found. In these 31 subjects with FVII deficiency, the mean FVII level was 54.5% ±8.44 (range, 39-69%).

As to natural anticoagulant levels, whereas no case of antithrombin deficiency was found, protein C deficiency was present in 34 subjects (32.5% of GBP patients *vs* 11.4% of SG patients, p=0.033) and protein S deficiency in 39 (37.6% of GBP patients *vs* 12.6% of SG patients, p=0.009). In the deficient subjects, the mean level of protein C was 57.4% ±4.58 (range, 50-65%) and the mean level of protein S was 63.1% ±3.79 (range, 58-69%).

Separate multivariate analyses confirmed that GBP was associated with an increased risk of deficiency in FVII (odds ratio [OR]: 3.64; 95% confidence interval [CI]: 1.73-7.64; p=0.001), protein C (OR: 4.319; 95% CI: 1.33-13.9; p=0.015) and protein S (OR: 5.50; 95% CI: 1.71-17.7; p=0.004).

Discussion

Results of this prospective study provide evidence about the effects of bariatric surgery on haemostatic and fibrinolytic balance. In detail, we documented that bariatric surgery is able to reduce the hypercoagulable state typical of obese subjects. Although both SG and GBP showed a clear efficacy in reducing levels of clotting factors and fibrinolytic variables, GBP was associated with greater reductions in FVII, protein C and protein S as compared with those following SG. However, these findings need to be discussed thoroughly.

The total fibrinolytic potential of human blood is determined by the balance between plasminogen activators (t-PA) and plasminogen activator inhibitors (PAI-1)[16]. Increased PAI-1 levels are the expression of impaired fibrinolysis and, by inducing a pro-coagulant shift in the haemostatic balance, are associated with fibrin generation and, in turn, thrombosis[17]. Several studies have already documented impaired fibrinolysis in obese subjects and also demonstrated the effects of weight loss on reducing PAI-1 levels[18]. Our study, by showing a correlation between changes in BMI and in PAI-1 induced by bariatric surgery, confirmed and extended these data.

As to t-PA, the major activator of fibrinolysis, this has often been found to be increased in hypercoagulable states, in an attempt to counterbalance the thrombotic tendency[4]. Interestingly, in the present study we confirmed a direct correlation between post-operative Δ%PAI-1 and Δ%t-PA, further supporting this hypothesis.

Overall, our data showed that SG and GBP had similar efficacy in improving fibrinolytic balance, being associated with an approximately 20% reduction in PAI-1, which was accompanied by an approximately 10% reduction in t-PA.

Moving to the other haemostatic variables, compared to SG, GBP was associated with greater reductions in

FVII, protein C and protein S levels. More in detail, changes in these three parameters seem to have different mechanisms and to exceed physiological changes. Indeed, besides weight loss-induced reductions, we also identified some cases of deficiency in these three haemostatic factors.

It is interesting to highlight that all three of these factors are vitamin K-dependent proteins. Vitamin K is one of the fat-soluble vitamins usually absorbed in the proximal small intestine by a saturable energy-dependent process[19]. Two other vitamin K compounds (menaquinone and menadione) are absorbed by passive diffusion in the distal intestine and colon. As a consequence, pathological conditions in which there is malabsorption (e.g., Crohn's disease) may be associated with vitamin K deficiency[19].

The risk of malabsorption and vitamin K deficiency after bariatric surgery is significant, being reported in to occur in 20% of patients after GBP[20]. Indeed, most of the malabsorptive bariatric procedures (such as GBP) involve surgical exclusion of a significant portion of bowel, usually including the proximal small intestine, which is one of the major sites of vitamin K absorption.

A lack of vitamin K can be associated with deficiencies in vitamin K-dependent clotting factors[21]. Although vitamin K-deficiency related changes in clotting factors have been thought to increase the risk of bleeding in patients who undergo bariatric surgery[22], it is important to highlight that data derived from studies on major inherited bleeding disorders (haemophilia A and B, FVII deficiency)[23,24] clearly indicate that mild deficiencies (clotting factor levels ~40%) in FVII, FVIII and FIX are not associated with an increased risk of bleeding. Indeed, in haemophilia patients and in patients with FVII deficiency, bleeding manifestations usually occur when clotting factor levels are lower than 10%-20%. In our population, the 31 patients with FVII deficiency had mean levels of approximately 50% and no unexpected perioperative bleeds were reported in our patients. Thus, although GBP is associated with a higher risk of acquired FVII deficiency, the bleeding risk should be marginal. However, further studies designed to address this issue are needed.

Moving to natural anticoagulants the situation is totally different. Antithrombin, protein C and protein S are major compounds of the physiological anticoagulant system and their deficiencies are known to be severe risk factors for venous thromboembolism[25]. Indeed, some recent data clearly demonstrated that even a mild deficiency (factor levels 70-80%) of antithrombin, protein C or protein S is associated with an increased risk of thrombosis[12,26,27].

In the present study we have found a deficiency of protein C and protein S in 21.8% and 25% of patients, respectively. In contrast, no alterations in antithrombin levels were found. This is in line with the hypothesis of vitamin K deficiency-related alterations, since protein C and protein S are synthesised through vitamin K-dependent mechanisms, while the production of antithrombin is totally independent of this compound. Although vitamin K levels were not assessed in this study, the hypothesis of a vitamin K deficiency-dependent mechanism is supported by the fact that deficiencies in protein C and protein S are more frequent in patients who undergo GBP, which is a malabsorptive procedure, than in those who undergo SG, which is a technique designed to preserve physiological gut absorptive function[28]. However, in line with previous studies showing that micronutrient deficiencies can also occur in patients undergoing restrictive procedures[29,30], we also found some patients with clotting factor deficiencies in the SG group. This could be secondary to persistent vomiting and, in turn, to impaired absorption of macro- and micro-nutrients.

Some limitations of this study should be discussed. Most of reported changes occurred in vitamin K-dependent factors and pre- and post-operative evaluation of vitamin K levels could have been useful to extend our findings. Although these data were not available for our patients, a previous study has already shown that the prolongation in activated partial thromboplastin time found in some patients undergoing bariatric surgery could be reverted by vitamin K administration[31].

Conclusion

In conclusion, our data suggest that although bariatric surgery is able to revert the hypercoagulable state usually reported in obese patients, in some cases, an acquired natural anticoagulant deficiency may occur and this could be associated with an increased risk of thrombosis[32]. Thus, changes in vitamin status and in haemostatic variables should be strictly monitored after bariatric surgery, particularly when malabsorptive procedures are used.

Authorship contributions

RL and MNDDM conceived and designed the study, performed the statistical analysis, interpreted the results and drafted the manuscript; MMi, ADM, PM, PA, MMu and RL acquired clinical data and drafted the manuscript.

RL and MMi contributed equally to this paper.

MNDDM and MMu share co-seniorship of the manuscript. All Authors read and approved the final version of the manuscript.

RL had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

The Authors declare no conflicts of interest.

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Correspondence: Matteo Nicola Dario Di Minno
Regional Reference Centre for Coagulation Disorders
"Federico II" University
Via S. Pansini 5
80131 Naples, Italy
e-mail: dario.diminno@hotmail.it