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TESI DI DOTTORATO

IMPACT OF BARIATRIC SURGERY ON CARDIOMETABOLIC AND NUTRITIONAL STATUS: PROS AND CONS

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1. General introduction and outline of the thesis

The epidemic spreading of obesity that currently affects both developed and developing countries is well defined by the term "globesity" [1,2,3]. In 2008, according to the World Health Organization (WHO), 1.4 billion adults, 20 years of age and older, were overweight with an estimated 500 million adults worldwide being obese (over 200 million men and nearly 300 million women) [2,3,4]. Noteworthy, the prevalence of obesity has tripled since the 1980s in many countries of the WHO European Region with overweight and obesity affecting 50% of the population in the majority of European countries [2,3,4]. If current trends continue, almost 60% of the world's population will be overweight (2.2 billion) or obese (1.1 billion) by 2030 [5]. Obesity is often associated with severe and progressive complications, such as cardiovascular events, hypertension, dyslipidemia, hyperuricemia, sleep apnoea, cancer, osteoarthritis, depression, infertility. Moreover, the lifespan of severely obese individuals is decreased by an estimated 5 to 20 years depending on gender, age and race [6]. Indeed, body mass index (BMI) is an independent risk factor for premature mortality [7] and for the development of type 2 diabetes mellitus (T2DM) and is also associated with the rapid increase of its incidence [8]. It was estimated that for every 1 kg increase in body weight, the risk of diabetes grows from 4.5 to 9% [9]. In the majority of European countries, overweight and obesity are responsible for about 80 % of cases of type 2 diabetes [4]. The term "diabesity" expresses the causal relationship between the two syndromes.

Bariatric surgery is considered an effective therapeutic option for obese patients to achieve a significant and sustained reduction in body weight with improvement or remission of the associated co-morbidities and, in particular, of

the metabolic ones [10,11,12,13,14,15]. In addition, bariatric surgery is proven to reduce overall and cardiovascular mortality [16,17,18] and to determine an overall improvement of the quality of life [19]. In the last decade, increasing evidence has shown that bariatric surgery is able to improve glucose metabolism or even reverse T2DM. For these reasons, it is considered an effective tool for the treatment of diabesity.

The present project was designed to assess the impact of bariatric surgery on clinically-relevant outcomes and to highlight the pros and cons of this therapeutic tool on specific metabolic, cardiovascular and nutritional aspects.

2. Overview of bariatric procedures

2.1 Indications to bariatric Surgery

According to the recommendations of the National Institutes of Health Consensus Conference (1991), obese patients with Body Mass Index (BMI) ≥40 kg/m^2 or >35 kg/m^2 in the presence of co-morbidities, who has failed repeated dietary and behavioral approaches and/or drugs for treatment of obesity, are candidates for bariatric surgery. These recommendations focusing on BMI as a principal determinant of mortality in obese patients has revealed important limits [(20)]. Indeed, these indications do not take into account the distribution of fat (somatic or visceral), a key factor in determining the metabolic syndrome, and the different distribution of fat in relation to age, gender and race.

For this reason, BMI is considered an important benchmark, but not the only one to establish the indication for bariatric surgery.

Moreover, bariatric surgery has revealed to be effective in patients with class 1 obesity (BMI between 30 and 35 kg/m^2) and obesity-related comorbidities. To date, there is growing evidence on the usefulness of the bariatric approach in patients with class I obesity. Indeed, randomized-controlled trials, prospective/retrospective studies and meta-analyses on class I obesity patients show the effectiveness of bariatric surgery in terms of both weight reduction and improvement of comorbidities [(21,22,23,24,25,26)].

On the basis of these data, BMI has to be evaluated together with metabolic, functional and psychological parameters for an overall balance between risks and benefits.

Moreover, the choice of the surgical treatment has to take into account the presence of absolute and relative contraindications, reversible or irreversible, in

the psychological/psychiatric field. In particular, according to current Italian guidelines [27]:

- anxiety disorder and/or depression are considered negative predictors for the outcome of bariatric surgery but not an absolute contraindication if associated with a psychiatric therapy;
- active bipolar disorder, schizophrenia, psychosis, alcohol addiction and bulimia nervosa are absolute contraindications;
- patients with Binge Eating Disorder or Night Eating Syndrome has indication for bariatric surgery only after multidisciplinary evaluation and psychotherapeutic treatment.

2.2 Surgical Techniques

Bariatric surgical procedures (Figure 1) can be classified on the basis of their mechanism of action in: restrictive, malabsorptive and restrictive-malabsorptive.

Restrictive procedures

Restrictive procedures promote weight loss by reducing gastric capacity through the creation of a small gastric pouch whose wall mechanoreceptors are stimulated by the food, thus activating the hypothalamic centers deputies to decreased appetite [28]. The maintenance of the sense of satiety depends on the degree of gastric restriction and, in turn, on the gastric wall stretching. Correct eating behavior is fundamental not only to achieve the sense of satiety, but also for the maintenance of the restriction volume and therefore of weight loss.

- ***Adjustable Gastric Band***

Adjustable Gastric Banding (AGB) consists of the placement of an inflatable silicone band around the upper portion of the stomach, creating a small proximal gastric pouch (about 30 ml) above the band. This procedure is minimally invasive and has the advantage to be reversible because of the ability of the prosthesis to tighten or widen the passage between the proximal and distal stomach by the insufflation of the band.

- ***Sleeve Gastrectomy***

SG is a bariatric technique consisting of subtotal vertical gastrectomy with preservation of the pylorus, including longitudinal resection of fundus, corpus and antrum, to create a tubular duct along the lesser curvature. Resection comprises approximately 80% of the stomach and the remnant gastric has a capacity > 100 mL.

Sleeve Gastrectomy (SG) or vertical gastrectomy consists of a subtotal vertical gastrectomy along the greater curvature with longitudinal resection of gastric fundus, corpus and antrum. It creates a "sleeve" along the lesser curvature with preservation of the pylorus. Resection comprises approximately 80% of the stomach and the remnant gastric has a capacity > 100 mL.

This procedure is not reversible. The mechanism of action does not involve only volume restriction but it encompasses a series of more complex neural/hormone mechanisms .

Unlike other restrictive techniques such as AGB, SG accelerates gastric emptying [(29)] and intestinal transit [(30)]. It seems that the rapid transit may trigger hormonal changes (in GLP-1, PYY, Ghrelin) that may contribute to weight loss [(31)].

Malabsorptive procedures

Malabsorptive procedures enhance weight loss by altering the structure of the digestive tract, allowing food to bypass portions of the small intestine. The only strictly malabsorptive weight loss surgery is biliopancreatic diversion.

- ***Biliopancreatic diversion***

Biliopancreatic diversion (BPD) was originally described by Scopinaro in 1979 as an alternative to jejunoileal bypass for severely obese patients. The BPD procedure consists of: a) partial distal gastrectomy in which the duodenal stump is closed (or bypass of the distal part of the stomach); b) transection of the small bowel approximately halfway between the ligament of Treitz and the ileocecal valve; c) Roux-en-Y gastroenterostomy from the gastric pouch to the distal bowel loop creating an alimentary limb; d) a biliopancreatic limb anastomosed with the alimentary limb 50 cm before the ileocecal valve forming a common channel [(32)]. The addition of the duodenal switch (DS), in which a vertical sleeve gastrectomy is combined with a duodenoenterostomy, was termed the "second generation BPD". DS involves preservation of the lesser curvature, antrum, pylorus, and first part of the duodenum along with lengthening of common channel lengths from 50 cm to 100 cm or more. These modifications were created to control for complications associated with Scopinaro's original description including marginal ulceration, vomiting, diarrhea, dumping syndrome, and micronutrient deficiencies. Indeed, after BPD the food does not mix with the bile and pancreatic enzymes until very far down the small intestine. This results in a significant decrease in the absorption of calories and nutrients (particularly protein and fat) as well as nutrients and vitamins dependent on fat

for absorption (fat soluble vitamins and nutrients). For all this reasons, BPD/DS is one of the most complex and highest risk bariatric surgeries utilized today.

Restrictive-malabsorptive procedures

Restrictive-malabsorptive procedures associate the bypass of small bowel to the reduction of gastric volume in order to maximize their effects on body weight while reducing the complications intrinsic to purely malabsorptive approach.

- ***Roux-en-Y Gastric Bypass***

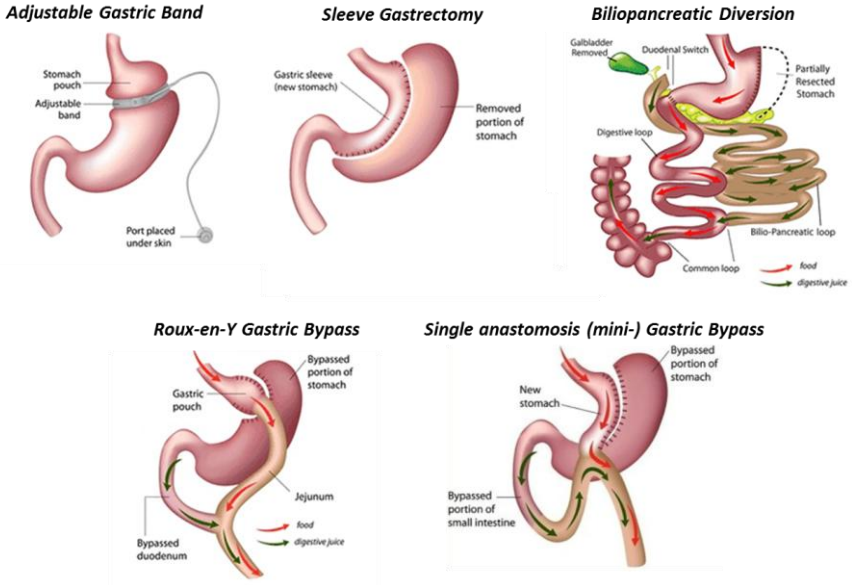
Roux-en-Y Gastric Bypass (RYGB) consists of the creation of a small gastric pouch (15–30 mL) on the lesser gastric curvature [33,34] which is completely divided from the gastric remnant and then anastomosed to the jejunum. Bowel continuity is restored by an entero-entero anastomosis between the excluded biliopancreatic limb and the alimentary limb. This anastomosis is usually performed 100–150 cm distal to the gastro-jejunostomy. The RYGB works by several mechanisms. First, similarly to most bariatric procedures, the newly created stomach pouch is considerably smaller and facilitates significantly smaller meals, which translates into less calories consumed. Additionally, because there is less digestion of food by the smaller stomach pouch, and there is a segment of small intestine that would normally absorb calories as well as nutrients that no longer has food going through it, there is less absorption of calories and nutrients.

Most importantly, the rerouting of the food stream produces changes in gut hormones that promote satiety, suppress hunger, and reverse one of the primary mechanisms by which obesity induces type 2 diabetes [35].

- ***Single anastomosis (mini-) gastric bypass***

Laparoscopic single anastomosis (mini-) gastric bypass (SAGB) creates a small gastric pouch of approximately 60 ml excluded from the rest of the stomach and connected to the small bowel by end-to-side anastomosis at a distance from the duodenum, not completely standardized, which normally corresponds to about 200 cm [(36)]. This is a key element, since in this way the distance of the anastomosis can vary from 600 to 200 cm, giving the intervention a malabsorptive connotation almost unpredictable [(27)].

Figure 1. Most common bariatric procedures.



2.3 Epidemiology of bariatric procedures

Between 150,000–200,000 bariatric procedures are performed annually in the U.S., and approximately 250,000 are performed outside the U.S. [(37)]. SG and RYGB are the most performed bariatric procedures. In particular, the frequency of use of the different procedures is: SG 49%, RYGB 43%, AGB 6%, and BPD+DS 2% [(38)]. SG has only recently replaced RYGB as the most common procedure worldwide, while AGB has steadily declined in usage over the past 5–8 years [(38)].

3. Impact of bariatric surgery on clinical outcomes: literature data

3.1 Effect on body weight

According to the current literature, bariatric surgery is the most effective weight-loss therapeutic option in terms of both magnitude of weight loss and durability. Weight loss is usually expressed as either percent weight loss ([weight loss in kg/initial weight in kg] X 100%) or percent excess weight loss (EWL) ([initial weight in kg - final weight in kg]/[initial weight - ideal body weight in kg] X 100%) [(39)]. Buchwald et al. in a meta-analysis on 136 studies (on 22 094 patients) showed a mean percentage of EWL of 61.2% (58.1%-64.4%) for all patients; 47.5% (40.7%-54.2%) for patients who underwent AGB; 61.6% (56.7%-66.5%) for RYGB; 68.2% (61.5%-74.8%) for gastroplasty; and 70.1% (66.3%-73.9%) for BPD [(40)]. As to SG, the average EWL is 50–55%, covering an intermediate position [(41)]. Data from Swedish Obese Subjects (SOS) study [(12)], one of the largest and longest prospective studies, showed at 20 years follow-up a mean percent weight loss of 26% for gastric bypass and 13% for gastric banding compared with 1% for control subjects. By contrast, long-term medical (nonsurgical) weight loss rarely exceeds 5% [(42)]. Moreover, also the expected percentage regaining weight back to baseline changes on the basis of the procedure performed: 18% for AGB, 5% for SG, and 2% for RYGB [(43)].

3.2 Remission of type 2 diabetes

Pories et al. in 1987 [(44)] published the interesting observation that almost all (99%) diabetic patients with severe obesity undergoing gastric bypass, achieve normalization of blood glucose levels. This effect occurs very early (few days after surgery). Subsequently, it was shown that both malabsorptive and restrictive procedures were able to improve glycemic control. Data from a meta-

analysis on 621 [45] studies for a total of 135,246 patients, showed that the effect of bariatric surgery on the improvement/remission of diabetes is very different depending on the surgical technique performed: 94% in patients undergoing BPD, 81.6% with RYGB and 55% with AGB. The SOS study reported a T2DM remission rate of 72% at 2 years and 36% at 10 years compared with 21% and 13%, in non operated, control subjects (P<0.001) [10]. The same study reported a 50% reduction in the chronic diabetic complications after surgery [46]. At 15 years follow-up, the cumulative incidence of microvascular complications was 41.8 per 1000 person-years (95% CI, 35.3-49.5) for control patients and 20.6 per 1000 person-years (95% CI, 17.0-24.9) in the surgery group (hazard ratio [HR], 0.44; 95% CI, 0.34-0.56; P < .001). Macrovascular complications were observed in 44.2 per 1000 person-years (95% CI, 37.5-52.1) in control patients and 31.7 per 1000 person-years (95% CI, 27.0-37.2) for the surgical group (HR, 0.68; 95% CI, 0.54-0.85; P = .001) [46]. With regard to randomized-controlled trials (RCTs), a recent meta-analysis of RCTs comparing bariatric surgery with medical treatment of T2DM [43] has shown the net superiority of bariatric surgery despite some variability in study design and patients characteristics among studies. Indeed, all but 1 study [47],showed that surgery was superior to medical treatment with respect to the primary end point (P<0.05 for all) (Figure 2). In particular, HbA1c decreased by 2%–3.5% with surgery and 1–1.5%, with medical treatment.

Figure 2. Panel A: Characteristic of RCTs – Panel B: Forest plot of mean differences (MDs) of %HbA1c serum levels after bariatric/metabolic surgery compared with medical/lifestyle treatments in published RCTs [43].

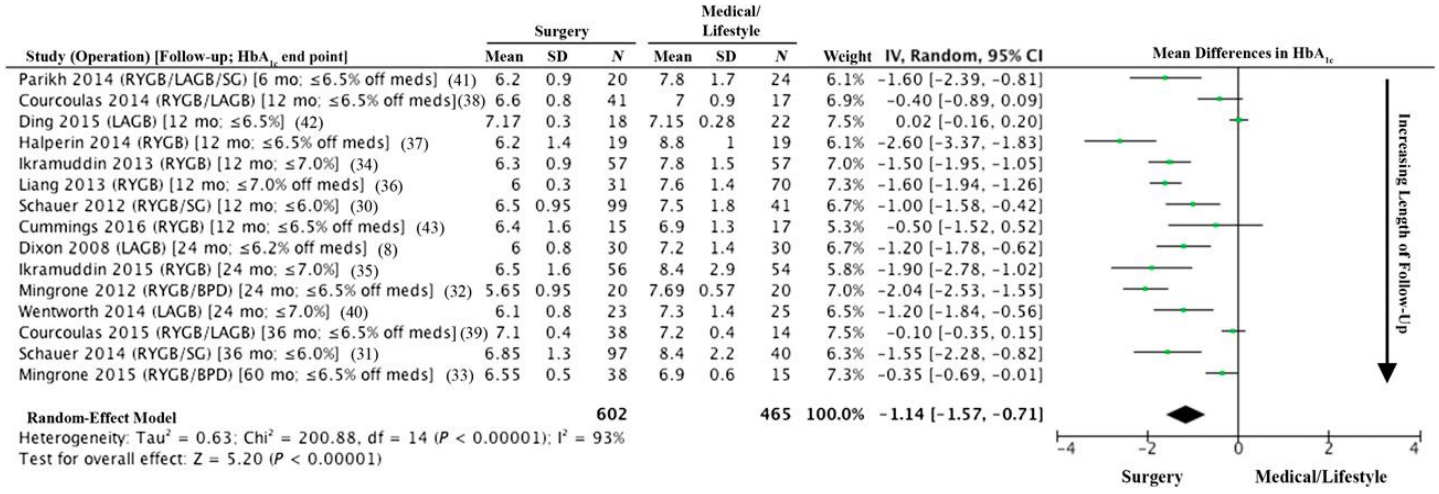
A

Table 1—Metabolic surgery RCTs for T2D (*n* = 794)

Study	BMI (kg/m^2), % of patients	Design	No. of patients randomized	Follow-up (months)	Remission criteria*	Outcome (remission or change in HbA_{1c})
Dixon (8)	<35, 22%	LAGB vs. control	60	24	HbA_{1c} <6.2%	73% vs. 13%, $P < 0.001$
Schauer (30,31)	<35, 36%	RYGB vs. SG vs. control	150	36	HbA_{1c} ≤6.0%	35% vs. 20% vs. 0, $P = 0.002$
Mingrone (32,33)	>35, 100%	RYGB vs. BPD vs. control	60	60	HbA_{1c} ≤6.5%	42% vs. 68% vs. 0, $P = 0.003$
Ikramuddin (34,35)	<35, 59%	RYGB vs. control	120	24	HbA_{1c} <6%	44% vs. 9%, $P < 0.001$
Liang (36)	<35, 100%	RYGB vs. control	101	12	HbA_{1c} <6.5%**	90% vs. 0 vs. 0, $P < 0.0001$
Halperin (37)	<35, 34%	RYGB vs. control	38	12	HbA_{1c} <6.5%	58% vs. 16%, $P = 0.03$
Courcoulas (38,39)	<35, 43%	RYGB vs. LAGB vs. control	69	36	HbA_{1c} <6.5%	40% vs. 29% vs. 0, $P = 0.004$
Wentworth (40)	≤30, 100%	LAGB vs. control	51	24	Fasting blood glucose <7.0 mmol/L	52% vs. 8%, $P = 0.001$
Parikh (41)	<35, 100%	Bariatric surgery (RYGB, LAGB, SG) vs. control	57	6	HbA_{1c} <6.5%	65% vs. 0, $P = 0.0001$
Ding (42)	<35, 34%	LAGB vs. control	45	12	HbA_{1c} <6.5%***	33% vs. 23%, $P = 0.46$
Cummings (43)	<35, 25%	RYGB vs. control	43	12	HbA_{1c} <6.0%	60% vs. 5.9%, $P = 0.002$

*Remission was a primary or secondary end point. Reaching HbA_{1c} value without diabetes medication, unless otherwise specified. **Remission not precisely defined, HbA_{1c} <6.5% by extrapolation. ***On or off diabetes medications.

B



Although physiological mechanisms behind T2DM remission are not completely understood, some evidence supports a key role of the gut hormones in the improvement of glucose homeostasis after bariatric surgery. Ghrelin, peptide YY, glucose-dependent insulinotropic peptide (GIP), and glucagon-like peptide 1 (GLP-1) are the gut hormones of particular interest. GLP-1 and GIP, known as incretins, are gastrointestinal hormones that stimulate postprandial β-cell insulin release, inhibit glucagon, slow gastric empting, and promote weight loss. GLP-1 is co-secreted with peptide YY by enteroendocrine L cells of the ileum and colon in response to a carbohydrate load. RYGB patients demonstrate an immediate 3- to 5-fold increase in postprandial GLP-1 and peptide YY levels postoperatively, which precedes significant weight loss and is independent of caloric restriction [(48)]. Restored GLP-1 levels may contribute to the recovery of early phase insulin secretion in response to oral carbohydrates. With regard to Ghrelin response after RYGB, the data of the literature are quite heterogeneous and therefore its contribution to the reduced appetite and improved glucose homeostasis remains unclear [(49)].

Two hypotheses have been proposed to explain the changes in gut hormones levels after bariatric surgery [(50)]. The "hindgut hypothesis" suggests that the quick transit of nutrients to the distal bowel improves glucose metabolism by stimulating secretion of GLP-1 and other appetite-suppressing gut peptides. The "foregut hypothesis" proposes that the exclusion of the duodenum and proximal jejunum from the transit of nutrients may prevent the secretion of yet unknown factor(s) that promote(s) insulin resistance and type 2 diabetes mellitus.

Further studies are needed to clarify the exact role of gut hormones in T2DM remission after bariatric surgery and to highlight any procedure-specific effect on their secretion pattern.

3.3 Decrease in cardiovascular risk and mortality

The overall impact of bariatric surgery on cardiovascular (CV) risk factors is well documented in a recent systematic review [51]. Across 19,543 surgical subjects with a mean follow-up period of 57.8 months, the average EWL for all procedures was 54% and hypertension remitted or resolved in 63% of affected subjects, dyslipidemia in 65%, and diabetes mellitus in 73%. The favorable effect of bariatric surgery on CV risk factors translates into reduced CV mortality, as shown by Vest et al. [52] (Table 1). Despite the absence of RCTs, a series of large studies [53,16,54] comparing post-bariatric patients with matched nonsurgical controls, consistently showed a 40%-60% higher survival in surgical patients versus nonsurgical control subjects. At 14.7 year follow-up of the SOS study [12]. Cardiovascular mortality in the surgical group was significantly lower than that of control subjects (adjusted hazard ratio, 0.47; 95% CI, 0.29– 0.76; P=0.002) despite the greater prevalence of smoking and higher baseline body weight and blood pressure in the surgical cohort. This finding has been confirmed and extended in a recent meta-analysis [55] of 14 studies: compared to nonsurgical controls, there was more than 50% reduction in mortality among bariatric patients (OR 0.48 95% CI 0.35-0.64, I^2=86%). In pooled analysis of 4 studies with adjusted data, bariatric surgery was associated with a significantly reduced risk of composite cardiovascular adverse events (OR 0.54 95% CI 0.41-0.70, I^2=58%).

Table 1. Major Studies of Bariatric Surgery With Cardiovascular Event or Mortality End Points [52].

Authors, Year	Follow-up Period	Outcomes
MacDonald et al.,1997 [56]	9 y (mean) for subjects, 6.2 y for control subjects	9% Mortality in subjects (including perioperative) vs 28% in control subjects (P<0.0003); annualized mortality rate of 1.0% in subjects vs 4.5% in control subjects
Christou et al., 2004 [57]	5y	0.68% Mortality in surgical group (including 0.4% perioperative mortality) vs 6.17% for control subjects (RR, 0.11; 95% CI, 0.04–0.27)
Flum and Dellinger, 2004 [53]	Median, 4.4 y; maximum, 15.5 y	At 15 y, 11.8% mortality in subjects vs 16.3% in control subjects; after propensity matching, odds of survival at 5 y, 59% higher in surgical group (OR, 1.59; 95% CI, 1.49–1.72)
Sampalis et al., 2006 [58]	5y	Decreased incidences of new pulmonary edema (RR, 0.42; 95% CI, 0.18–0.96), angina (RR, 0.53; 95% CI, 0.40–0.70), coronary artery bypass grafting (RR, 0.28; 95% CI, 0.14–0.61), and coronary angioplasty (RR, 0.36; 95% CI, 0.19–0.66)
Livingston et al., 2006 [59]	Maximum, 2 y	30-d cardiac arrest rate, 1.6%; 30-d myocardial infarction rate, 0.5%; overall 30-d mortality, 1.4%; and 2-y mortality, 3.1%
Adams et al., 2007 [54]	Mean, 7.1 y	lower in surgical group (adjusted HR, 0.60; 95% CI, 0.45–0.67; P<0.001); lower surgical mortality for all diseases combined (52%; P<0.001), CAD (59%; P=0.006), diabetes mellitus (92%; P=0.005), and cancer (60%; P=0.001)
Torquati et al., 2007 [60]	5 y	1% for CV event rate at 5 y
Sowemimo et al., 2007 [61]	9 y	2.9% Mortality in subjects vs 14.3% in control subjects; adjusted mortality, 82% lower in surgical subjects (HR, 0.18; 95% CI, 0.09–0.35; P<0.0001)
Busetto et al., 2007 [16]	5 y	Survival was 60% higher in surgical group (P=0.0004); on multivariate Cox analysis, adjusted mortality risk was 0.36 (95% CI, 0.16–0.80) in the surgical group
Peeters et al., 2007 [62]	Median, 4 y for surgical subjects; mean, 12 mo for control subjects	Surgical patients had a 72% lower risk of mortality, adjusted for sex/age/BMI, than control subjects (HR, 0.28; 95% CI, 0.10–0.85
Maciejewski et al., 2011 [63]	Mean, 6.7 y	2- and 6-y crude mortality significantly lower for surgical patients (2.2% vs 4.6% [P<0.001] and 6.8% vs 15.3% [P<0.001], respectively); significance of mortality benefit lost with propensity matching of 1694 patients (HR 0.83; 95% CI, 0.61–1.14)
Adams et al., 2012 [64]	6 y	2.9% Mortality (12 of 418) in the surgical cohort vs 3.3% and 0.9% mortality in control groups 1 and 2, respectively
Sjöström et al., 2012 [12]	Median, 14.7 y	Lower CV mortality rate in surgery group (adjusted HR, 0.47; 95% CI, 0.29–0.76; P=0.002); first-time CV events also lower in surgical group (adjusted HR, 0.67; 95% CI, 0.54–0.83; P<0.001)
Romeo et al., 2012 [65]	Mean, 13.3 y	Adjusted HR for myocardial infarction, 0.56 (95% CI, 0.34–0.93; P=0.025); adjusted HR for first-time CV event, 0.53 (95% CI, 0.35–0.79; P=0.002)

BMI, body mass index; CAD, coronary artery disease; CI, confidence interval; CV, cardiovascular; HR, hazard ratio; OR, odds ratio; RR, relative risk.

Moreover, bariatric surgery resulted to be associated with significant reduction in specific endpoints, such as myocardial infarction (OR 0.46 95% CI 0.30-0.69, I^2=79%, 4 studies) and stroke (OR 0.49 95% CI 0.32-0.75, I^2=59%, 4 studies).

3.4 Overall mortality and cancer outcomes

A further issue is whether bariatric surgery could improve other important long-term outcomes , such as overall mortality and cancer. To date, the findings in the published literature are inconsistent. Indeed, some evidence suggested potential benefits in the reduction of mortality and cancer [16,11] and some not [64,66]. A recent meta-analysis suggests that, compared to nonsurgical treatment, bariatric surgery could reduce all-cause mortality and the risk of cancer in obese patients. In particular, the analysis of 19 studies on overall mortality showed lower all-cause mortality in bariatric patients (1274/28,528 [4.5%] vs. 14,574/171,852 [8.5%], OR 0.38, 95%CI 0.29 to 0.50, I^2=91%) and the analysis of 6 studies on cancer outcomes showed statistically lower incidence of cancer in the bariatric group (OR 0.65, 95%CI 0.46 to 0.91, I^2=86%).

4. Personal research areas

4.1 Bariatric surgery and cardiometabolic effects

4.1.1 Glucose homeostasis and lipid metabolism

As reported above, RYGB and SG are the most widely performed bariatric procedures with a proven good clinical efficacy in obese patients with T2DM [37,43]. Previous studies have examined the effects of the two procedures with regard to diabetes remission, but limited information is available on the contribution of intervention-specific changes in gastrointestinal (GI) hormonal pattern to the remission of diabetes. To this end, we performed a prospective study [67] to compare the changes in insulin sensitivity, insulin secretion, and postmeal GI hormone levels in obese patients with T2DM 1-year after SG or RYGB, in order to evaluate the hormonal and metabolic mechanisms involved in weight loss and remission of T2DM.

The study group included 33 obese patients with T2DM (M/F: 14/19; mean age: 46±9 years, BMI: 44±8 kg/m^2), who were on a waiting list for bariatric surgery. In total, 14 subjects underwent RYGB and 19 subjects underwent SG. Antidiabetic treatment was oral hypoglycemic agents (OAD) in 24 patients, combined OAD plus bedtime insulin in 5 patients and diet alone in 4 patients. None was on multiple insulin injection regimen. Fourteen patients (74%) in SG and 9 (64%) in RYGB were on antihypertensive drugs; 5 patients (36%) in the RYBG group and 3 patients (16%) in the VSG group were on hypolipidemic treatment. Before and one year after the bariatric procedure, anthropometric, clinical and routine laboratory parameters were collected together with data on medication use. On both occasions, in the morning after a 12-h overnight fast, a standard glucose tolerance test (OGTT, 75g of glucose) was performed in order to evaluate insulin

secretion and insulin sensitivity. The day after, a liquid mixed-meal test (MMT) (304 kcal, containing 41 g carbohydrate, 13 g protein, and 9 g fat) was performed to evaluate GI hormonal response.

During MMT, blood samples were drawn at times 0, 30, 60, 90, 120, and 180 min for the measurement of glucose, insulin, active GLP-1 and total GIP concentrations at 0, 60, 120, and 180 min for the measurement of total ghrelin.

Insulin sensitivity and insulin secretion indexes were derived from glucose, insulin, and C-peptide values measured every 30min for 3h during the OGTT. Insulin sensitivity was assessed as the oral glucose insulin sensitivity (OGIS) [68]. Insulin secretion as total amount of the hormone released by the beta cells (ISR) was calculated from C-peptide with the deconvolution method [69]. Beta-cell function, which reflects the release of the hormone normalized to the glycemic stimulus, was assessed as "early" (IGI30= ratio between incremental C-peptide concentration and incremental glucose concentration at 30min) and "total" insulinogenic index (IGItotal= AUCCpeptide/AUCGlucose). The interplay between insulin sensitivity and secretion, that describes the beta-cell adaptive response to changes of insulin sensitivity, was determined by the product OGIS×AUCCpeptide (adaptation index, AI) [70].

Partial T2DM remission was defined as HbA1c <6.5% (47.5mmol/mol) and fasting glucose <126 mg/dl in the absence of antidiabetic medications. Complete remission was defined as HbA1c <6% (42.1mmol/mol) and fasting glucose <100 mg/dl in the absence of antidiabetic medications.

Main clinical and metabolic characteristics of participants before and one year after surgery are reported in Table 2. Age, BMI, duration of diabetes, glucose control, and lipid profile at baseline were similar between RYGB and SG groups.

At one-year, weight loss both expressed as percent change in BMI and expressed as EWL% was similar after the two interventions (p=0.546 and p=0.146 respectively). Glycemic control improved similarly in the two groups with a mean HbA1c reduction of 2-2.5% (18–26 mmol/mol) from baseline values. Fasting triglycerides levels fell markedly after both procedures; plasma total and LDL-cholesterol decreased after RYGB whereas they remained substantially unchanged after SG (Table 2).

Table 2. Clinical and metabolic characteristics of participants before and one year after surgery.

	RYGB (n=14)			SG (n=19)			RYGB vs SG
	presurgery	1 yr	*p value* baseline *vs* 1 yr	presurgery	1 yr	*p value* baseline *vs* 1 yr	*p value* 1 yr
Age (years)	49±7	---	---	44±10	---	---	---
Sex (M/F)	6/8	---	---	8/11	---	---	---
Weight (kg)	116±20	78±8	0.000	130±29	90±17	0.000	0.022
BMI (Kg/m^2)	42±6	28±2	0.000	46±9	32±5	0.000	0.009
EWL (%)	---	66±15	---	---	58±16	---	0.146
ΔBMI (%)	---	-32±10	---	---	-30±7	---	0.546
T2DM duration (years)	5±5	---	---	4±4	---	---	---
Glucose (mg/dl)	166±63	76±8	0.000	140±46	87±18	0.006	0.184
HbA_{1c} (mmol/mol)	65±1	39±0.1	0.003	60±0.8	38±0.1	0.004	0.759
Total cholesterol (mg/dl)	202±26	167±38	0.010	207±57	211±49	0.740	0.010
HDL-cholesterol (mg/dl)	44±8	51±10	0.009	44±10	59±16	0.004	0.089
LDL-cholesterol (mg/dl)	120±25	91±32	0.044	127±48	136±44	0.389	0.013
Triglycerides (mg/dl)	195±76	114±78	0.002	220±120	101±27	0.002	0.474
Therapy							
Diet alone (%)	2 (14)	13 (93)	0.017	2 (11)	17 (90)	0.003	0.800
OAD users n (%)	9 (64)	1 (7)	0.026	15 (79)	2 (10)	0.007	0.887
OAD+insulin users n (%)	3 (21)	0 (0)	0.098	2 (10)	0 (0)	0.167	0.809
Anti-hypertensive drug users n (%)	9 (64)	3 (21)	0.142	14 (74)	2 (10)	0.009	0.392
Hypolipidemic drug users n (%)	5 (36)	1 (7)	0.135	3 (16)	2 (10)	0.674	0.387

The remission of diabetes (partial plus total) was achieved in 14 SG patients (74%) and in 12 RYGB patients (86%) (p=0.28). Insulin secretion and insulin sensitivity (OGTT) Total insulin secretion (ISR) did not change, while beta-cell function improved to a similar extent one year after surgery (Table 3). Insulin sensitivity (OGIS) was similar in the two groups, preoperatively and markedly

improved after either procedures (p <0.001 for both). Adaptation index (AI) increased to a similar extent after surgery with no difference between RYGB and SG.

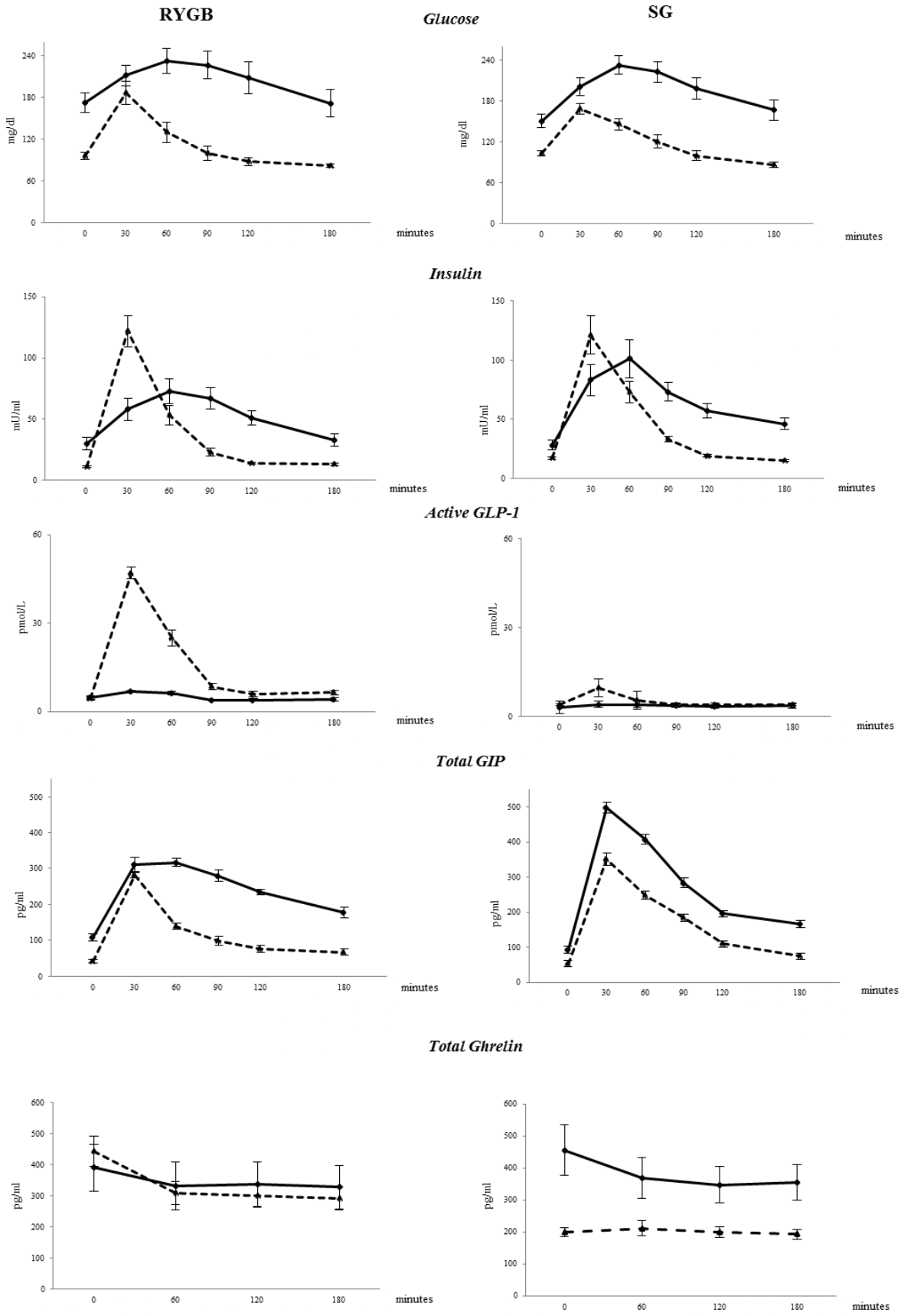
Table 3. Insulin sensitivity and insulin secretion, and glucose and hormonal response to MMT before and one year after surgery.

	RYGB (n=14)			SG (n=19)			RYGB vs SG
	presurgery	1 yr	Δ	presurgery	1 yr	Δ	pΔ
OGTT							
IGI 30 min (nmol/l/mg/dl)	0.2±0.1	0.5±0.2**	0.3±0.2	0.2±0.02	0.4±0.2*	0.2±0.2	**0.406**
IGI 180 min (nmol/l/mg/dl)	324±111	1603±1577*	1279±439	281±190	1059±952*	778±998	**0.424**
OGIS (ml min^{-1} m^{-2})	287±90	549±82**	262±88	302±49	500±86**	198±93	**0.177**
Adaptation Index	0.5±0.2	0.7±0.3*	0.2±0.2	0.4±0.2	0.7±0.3*	0.3±0.3	**0.390**
MMT							
Fasting Glucose (mg/dl)	173±50	96±16**	-76±50	151±46	104±18**	-47±39	**0.080**
IAUC Glucose (mg/dl · 180')	6246±5186	3016±3047	-3230±5710	8681±6678	3048±3490**	-5633±4999	**0.227**
Fasting Insulin (mU/ml)	30±18	11±4*	-19±17	28±16	17±6*	-11±17	**0.203**
IAUC Insulin (mU/ml· 180')	4313±2759	5118±2076	805±2808	7019±4343	5250±2866*	-1769±2959	**0.017**
Fasting GLP-1 (pmol/l)	4.7±4.5	4.5±3.7	-0.2±5.1	3.1±1.8	3.9±2.9	0.8±3.2	**0.579**
IAUC GLP-1 (pmol/l · 180')	27±130	2119±1200**	2092±1266	85±142	235±278	150±319	**0.000**
GLP-1 30-min peak (pmol/l)	7±6	47±22**	40±21	4±1	10±7*	6±7	**0.000**
Fasting GIP (pg/ml)	108±130	42±19*	-66±128	93±72	53±30*	-40±54	**0.501**
IAUC GIP (pg/ml · 180')	25344±15169	13234±4713**	-12110±15733	34132±11527	21871±5619*	-12261±9057	**0.977**
Fasting Ghrelin (pg/ml)	391±264	443±169	52±290	457±262	200±41*	-257±250	**0.013**
Ghrelin nadir (pg/ml)	309±72	278±38	31±73	334±64	167±12*	167±61	**0.035**

Glucose and hormone profile (MMT) $IAUC_{Glucose}$ decreased while $IAUC_{Insulin}$ increased after surgery with no difference between interventions (Figure 3).

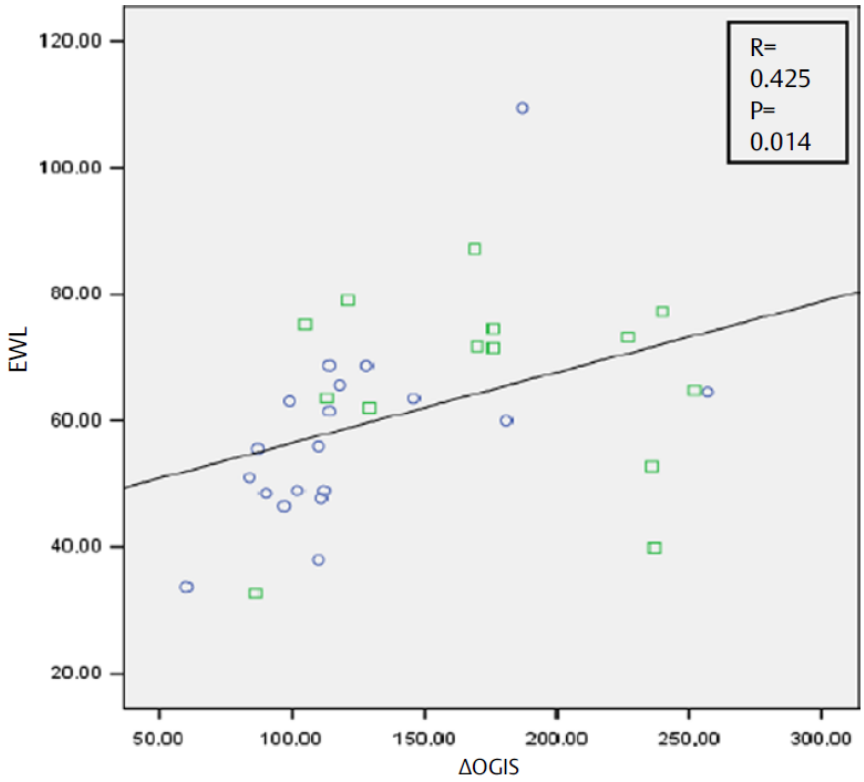
Meal-stimulated GLP-1 concentrations were flat in all patients preoperatively. Following RYGB, both GLP-1 peak and IAUC increased markedly (p = 0.001), while after SG, the release of GLP-1, although increased compared to presurgery, was much lower than in patients operated of RYGB (p = 0.0001). Meal GIP response after surgery decreased by 50 % (p = 0.001 after RYGB and p = 0.05 after SG) with no difference between interventions. Neither fasting nor nadir ghrelin during MMT changed after RYGB; in contrast, a marked suppression in both variables occurred after SG with a significant difference between the 2 intervention (p = 0.013 for fasting ghrelin and p = 0.035 for nadir ghrelin concentrations) (Figure 3 and Table 3).

Figure 3. Glucose, insulin, GLP-1, GIP, and ghrelin response to a mixed meal in RYGB and SG subjects before (continuous line) and one year (dotted line) after surgery.



The increase in insulin sensitivity and beta-cell function was correlated with weight loss (R = 0.425, p = 0.014 and R = 0.461, p = 0.035, respectively) (Figure 4) while no association was found with GI hormone concentrations.

Figure 4. Correletion between excess weight loss (EWL) and changes of insulin sensitivity (OGIS) in SG (circle) and RYGB (square) subjects.



In this study, we found that RYGB and SG resulted to be equally effective in terms of weight loss and improvement of glycemic control, with a similar rate of T2DM remission at 1 year (76 % after VSG and 86 % after RYGB); on the other hand, the two procedures are characterized by a different GI hormonal pattern.

This finding leads us to hypothesize that the changes in gut hormones are not the main determinant of the metabolic improvement, at least several months after surgery. However, since our evaluations were performed one year after surgery we cannot rule out that the changes in GI hormonal profile may have contributed to diabetes remission early after surgery. This hypothesis is in line with a recently

published commentary, which underlined that the mechanisms behind the remission of diabetes after SG or RYGB may differ in relation to the time at which they are studied. Early after surgery, the improvement of glycemic control is due to increased hepatic insulin sensitivity and to the improved beta-cell function consequent to the exaggerated postprandial GLP-1 secretion. Later on, with progressive weight loss the improvement in peripheral insulin sensitivity becomes the prevalent mechanism [71].

Besides glucose metabolism, there is clinical evidence that bariatric surgery exerts long-term favorable effects on fasting lipid levels with reduction of triglycerides and LDL cholesterol and increase in HDL cholesterol concentrations [72]. Moreover, although a number of studies have addressed incretin regulation of glucose homeostasis [73,74], much less is known about the relationship between incretin hormones and lipid metabolism and the effects of bariatric surgery on impairment of postprandial lipid profile, which is considered as an independent cardiovascular risk factor [75]. Accordingly, we performed a study [76] with the aim to evaluate the short-term (2 weeks) effects of bariatric surgery on fasting and postprandial lipid metabolism in obese T2DM patients and to establish whether changes in lipid profile are related to active GLP-1 changes.

Twenty-five obese T2DM2 patients (12 men and 13 women) were studied. Fifteen patients underwent SG, and ten underwent RYGB. All participants were studied before and 2 weeks after surgery. In both occasions, anthropometric, clinical, and laboratory parameters were collected together with data on medication use. In addition, plasma levels of glucose, insulin, lipids, and active GLP-1 were evaluated at fasting and after a standard MMT (composition reported above).

The clinical and metabolic characteristics of participants before and after surgery are given in Table 4. At week 2 after surgery, there was a significant reduction in body weight, fasting plasma glucose, and insulin as well as a marked reduction in insulin resistance, as evidenced by a 50% decrease in HOMA-IR. Glucose response to the MMT was significantly reduced compared to pre-intervention, while insulin response increased without reaching the statistical significance. The response of active GLP-1 to the MMT increased significantly after surgery.

Table 4. Clinical and metabolic characteristics of the patients before and two weeks after surgery.

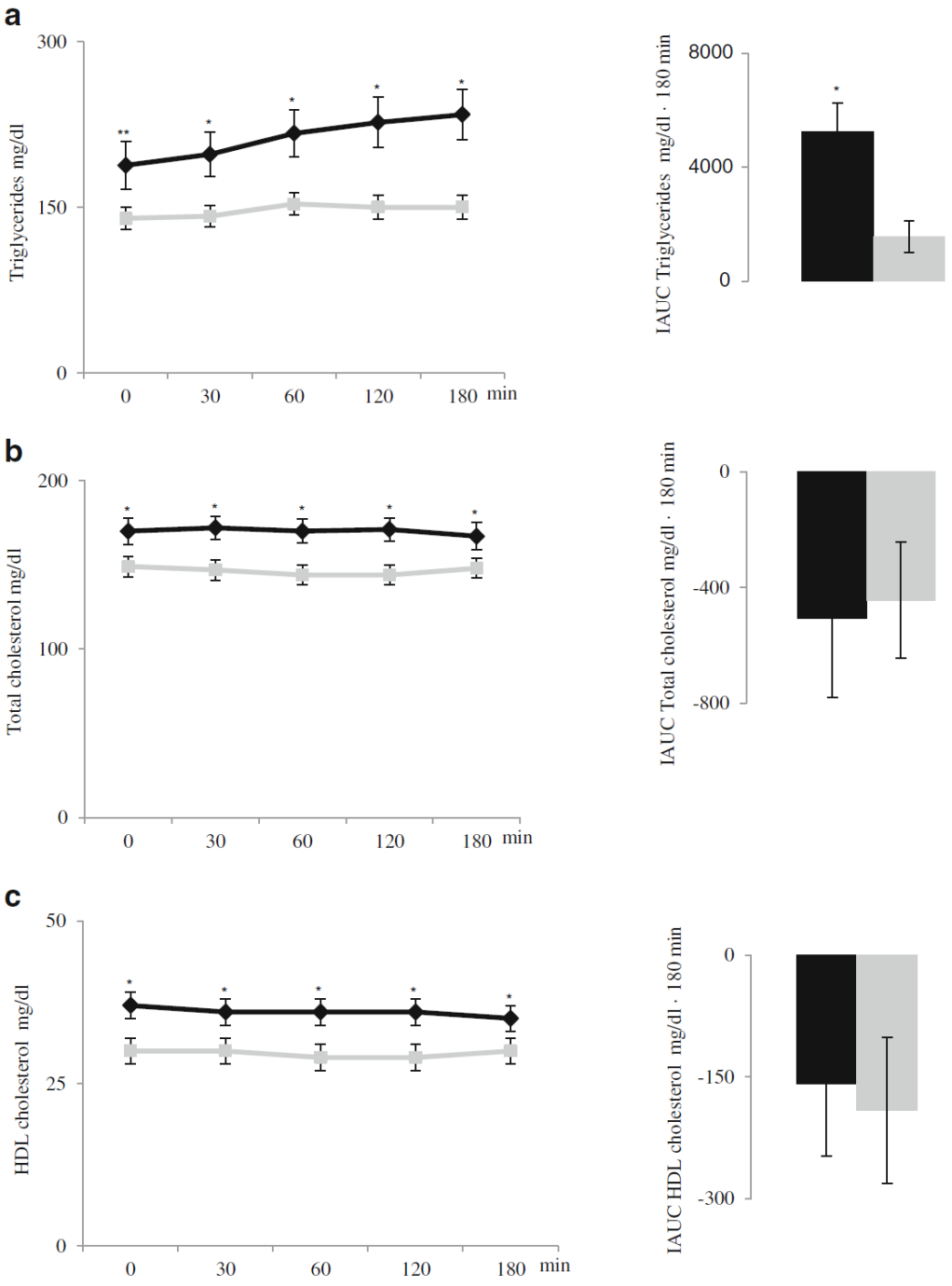
	Before surgery	After surgery	*p* value
Characteristic			
Male/female	12/13	–	
Age (years)	46±8	–	
Diabetes duration (years)	4±4	–	
Weight (kg)	124±23	110±22	0.000
BMI (kg/m^2)	44±7	39±7	0.000
HbA1c (%)	8±2	–	
Fasting glucose (mg/dl)	170±74	125±35	0.000
Fasting insulin (μU/ml)	25 (16, 35)	17 (12, 19)	0.004
HOMA-IR	9 (6, 14)	5 (4, 7)	0.000
Fasting GLP-1 (pmol/l)	3 (2, 4)	3 (2, 4)	0.977
Glucose IAUC (mg/dl)	8,867±5,733	5,744±4,676	0.019
Insulin IAUC (μU/ml·180 min)	5,757 (3,235, 7,513)	6,038 (2,766, 11,987)	0.110
GLP-1 IAUC (pmol/l·180 min)	43 (−13, 142)	1,136 (340, 2,455)	0.000
Therapy			
Diet	2/25	22/25	
Oral antidiabetic drugs	21/25	2/25	
Oral antidiabetic drugs plus insulin	2/25	0/25	
Insulin alone	0/25	1/25	
Antihypertensive drugs	15/25	0/25	

Data are means±SD or median and interquartile range (25, 75) BMI body mass index, IAUC incremental area under curve, HOMA-IR homeostasis model assessment of insulin resistance

The changes in lipid concentrations after surgery are reported in Figure 5. After surgery, there was a significant reduction in fasting plasma TG (182 mg/dl (110,

231) vs. 130 mg/dl (104, 165), p <0.05), total cholesterol (170±41 vs.148±29 mg/dl, p <0.005), LDL cholesterol (119±30 vs. 100±28 mg/dl, p <0.05), and HDL cholesterol (37±9 vs. 30±10 mg/dl, p <0.001).

Figure 5. Plasma concentration and IAUC of plasma triglycerides (a), plasma total cholesterol (b), and plasma HDL cholesterol (c) after mixed meal before (black diamond) and 2 weeks after (light grey square) bariatric surgery. Data are means±SE. * p <0.001 vs. 2 weeks; **p<0.05 vs. 2 weeks.



Post-MMT plasma TG, total cholesterol, and HDL cholesterol were significantly lower compared to pre-intervention. IAUC of plasma TG decreased markedly by 60 % (4,050 mg/dl·180 min (2,019, 8,409) vs. 1,635 mg/dl·180 min (390, −2,603), p <0.001), while no difference was observed in IAUC of total and HDL cholesterol (Figure 5). Analyzing postprandial lipid response according to the type of surgery (SG or RYGB), no difference was found between the two procedures.

In order to confirm and further extend these findings, we performed the same experimental evaluations in a subgroup of 19 patients (10 undergoing SG and 9 RYGB) with a 2-year post-surgery follow-up [77].

Postprandial triglycerides markedly decreased whereas HDL cholesterol increased after both interventions (Figure 6). Conversely, postprandial LDL cholesterol levels were significantly lower after RYGB compared to SG ($p<0.05$). No significant difference was found in the IAUC of triglycerides, HDL, and LDL cholesterol between the two interventions (Figure 6). GLP-1 meal response, very flat preoperatively, increased after surgery, with a higher increment after RYGB (IAUC 1753±271 vs 256±78 pmol/l×180 min, $p<0.001$). GLP-1 peak was 10±2 pmol/l after SG and 49±6 pmol/l after RYGB ($p=0.001$) (Figure 7). The decrease of fasting triglycerides was positively correlated with weight loss ($R=0.470$, $p=0.049$), reduction of HOMA-IR ($R=0.679$, $p=0.001$). In the RYGB group, LDL cholesterol was inversely related with GLP-1 peak ($R=-0.733$, $p=0.007$) adjusted for pre-surgery values. The multivariate model, adjusted for age, gender, duration of T2DM and HbA1c, showed that GLP-1 peak was the best predictor of LDL reduction ($\beta=-0.552$, $p=0.039$) while reduction of HOMA-IR ($\beta=0.574$, $p=0.014$) and weight loss ($\beta=0.418$, $p=0.036$) predicted triglycerides improvement.

Figure 6. Plasma concentration and IAUC of triglycerides, HDL cholesterol and LDL cholesterol after a mixed meal before (continuous line and white bar) and 2 years (dotted line and gray bar) after sleeve gastrectomy (SG) or gastric bypass (RYGB). Data are expressed as means (±SEM). *p<0.05, pre- vs post-surgery

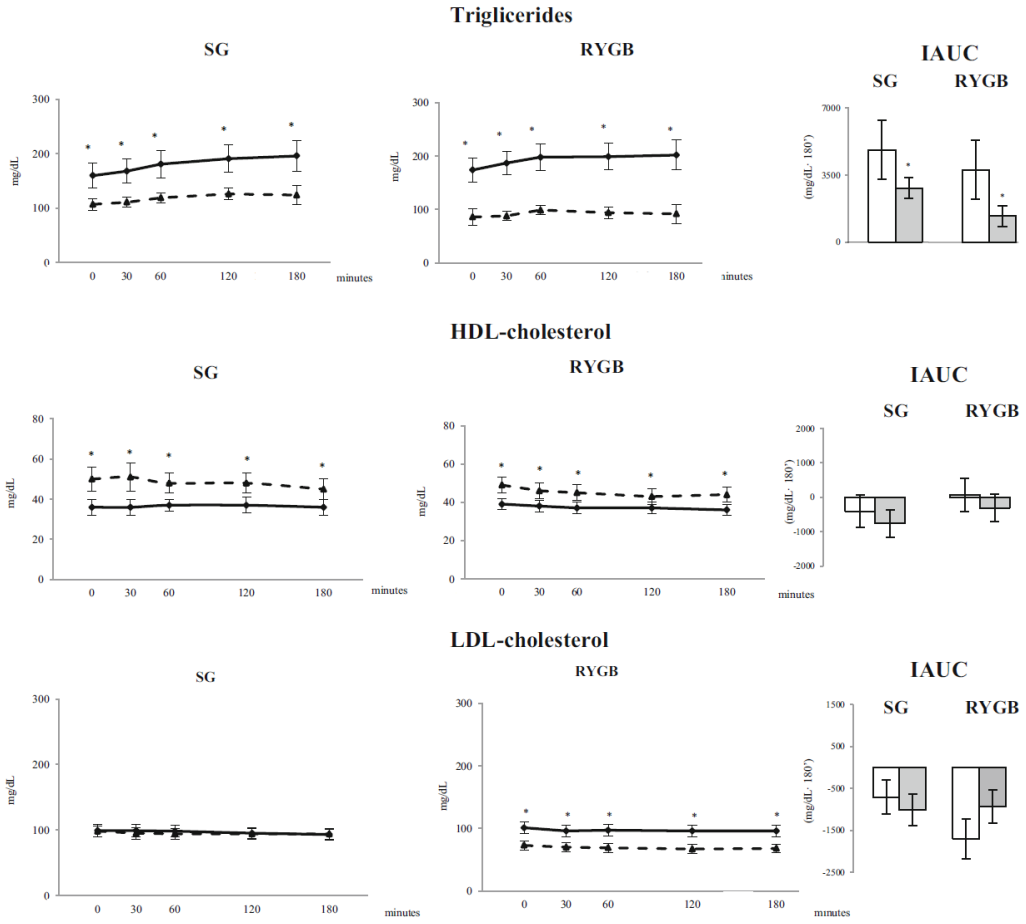
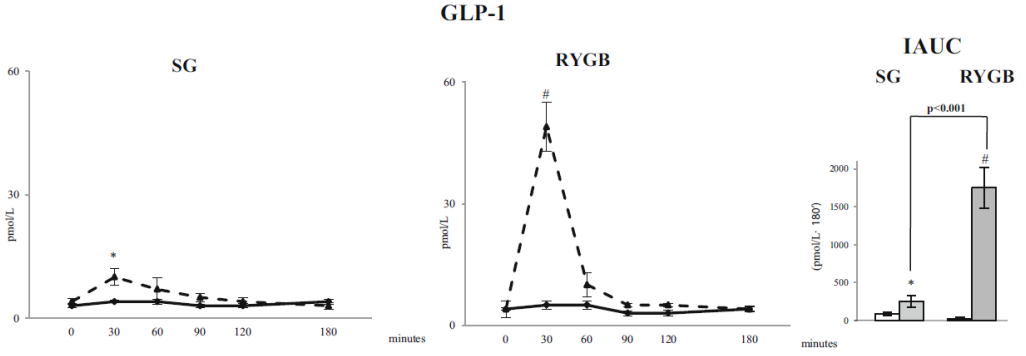


Figure 7. Plasma concentration and IAUC of GLP-1 after a mixed meal before (continuous line and white bar) and 2 years (dotted line and gray bar) after sleeve gastrectomy (SG) or gastric bypass (RYGB). Data are expressed as means (±SEM). *p<0.05 and #p<0.001, pre- vs post-surgery



Our data demonstrate that bariatric procedures, namely SG and RYGB, improve fasting as well as postprandial lipid profile. This effect occurs within 2 weeks after the intervention and persists up to 2 years after.

The major effect reported is the marked fall in postprandial triglycerides. This improvement of triglyceride metabolism was similar with both procedures, both in early and in late follow-up. On the other hand, LDL cholesterol levels resulted ~30 % lower after RYGB than SG 2 years after surgery. This finding is in agreement with recent evidence indicating that the type of surgery impacts primarily cholesterol metabolism rather than triglycerides. In fact, Benetti et al. reported a significant reduction in cholesterol levels after malabsorptive procedures (biliopancreatic diversion and biliointestinal bypass) but not after purely restrictive procedures (adjustable gastric banding), whereas triglycerides decreased similarly with the two types of surgery [78]. Moreover, in line with literature data [79,80], HDL cholesterol decreased a few days after bariatric procedures and increased later on during the follow-up. This finding is not unexpected because HDL cholesterol tends to decrease in highly dynamic conditions (marked reduction of food intake) [81], such as that immediately after bariatric surgery.

The significant inverse correlation between LDL cholesterol and GLP-1 meal response found in our RYGB patients supports the hypothesis that the restoration of GLP-1 may contribute to the reduction of plasma cholesterol level. In experimental animals, GLP-1 is able to suppress hepatic lipogenesis through activation of AMPK pathway [82] and the genes involved in fatty acid β-oxidation [83].

In conclusion, either RYGB and SG persistently improve glucose metabolism, reduce both fasting and postprandial triglycerides, and increase HDL cholesterol. LDL cholesterol decreases only after RYGB; this effect—likely mediated by surgery-induced GLP-1 restoration—underlines the crucial role of gut in the regulation of lipid metabolism.

4.1.2 Glucose variability and oxidative stress

Bariatric surgery causes a profound rearrangement of the gastro-intestinal anatomy that results, among others, in an accelerated gastric emptying [(84)]. It is well established that the rate of gastric emptying is a major determinant of postprandial glucose response both in healthy and in diabetic individuals [(85)]. The rapid entry of nutrients into the small intestine causes earlier and higher postprandial glycemic peaks followed by lower glucose nadirs, sometimes triggering frank hypoglycemic symptoms, as increasingly reported in recent studies [(86,87,88)]. These findings raise the issue as to whether and to which extent glucose homeostasis is normalized in diabetic patients undergoing bariatric surgery, and prompt for a more accurate assessment of glucose status in the postoperative follow-up. Among measures of glucose control, glycemic variability (GV), i.e. blood glucose oscillations throughout the day, is gaining increasing attention since high GV may be involved in the pathogenesis of diabetic vascular complications and mortality risk [(89,90,91,92)]. In the present study, we performed continuous glucose monitoring (CGM) on an ambulatory basis in patients who achieved diabetes remission after bariatric surgery in order to evaluate the pattern of glucose fluctuation under condition of real life. Since increased glucose variability activates oxidative stress [(93)], we also measured 24-h urinary excretion

of free 8-isoprostaglandin F2α (8-isoPGF2α), a well-recognized marker of oxidative stress.

The study groups included 22 patients (M/F:10/12; mean age: 50 ± 9 years, body mass index: 31 ± 6 kg/m^2) who were in remission of T2DM (T2DM remitters) after bariatric surgery since at least 1 year. Ten patients (45%) achieved complete remission (fasting plasma glucose <100 mg/dl, HbA1c <6% and no antidiabetic medication use) while 12 patients (55%) achieved partial remission (fasting plasma glucose between 100 and 126 mg/dl, HbA1C between 6 and 6.5% and no antidiabetic medication use). Eleven subjects had undergone RYGB and eleven SG. Twenty-two age-, sex- and BMI- matched subjects (M/F: 10/12; mean age: 52 ± 9 years, BMI: 32 ± 6 kg/m^2) recruited from the obesity outpatient clinic or the staff of Federico II University Hospital were enrolled as control. None of them had signs of liver, kidney or cardiac disease.

In all participants, a 75 g OGTT was performed after a 12-h overnight fast with sampling at 0', 30', 60', 90' and 120' for glucose and insulin measurement. Glucose and insulin responses to OGTT were calculated as the area under the curve above the baseline values using the trapezoidal method. The day before OGTT, they collected 24-h urinary samples for 8-isoPGF2α analysis.

After an overnight fast, all T2DM remitters and 10 control subjects underwent CGM for 7 days with survey of glucose levels in the interstitial fluid every 5 min, 270 times per day (Dexcom G4 PLATINUM). The application of the subcutaneous sensor was performed at the Outpatient Diabetic Clinic and after two hours the first calibration was carried out. Each participant was instructed to calibrate the CGM device twice daily or whenever alerted by the device. The download of glucose monitor data was performed using Dexcom StudioTM. GV

was analyzed with regard to the two principal components: amplitude and timing [94]. The amplitude was expressed by standard deviation of blood glucose (SD), coefficient of variation (CV), and mean amplitude of glucose excursions (MAGE) that was calculated as the arithmetic mean difference between consecutive blood glucose peaks and nadirs (between the peaks) when differences were >1 SD of the mean glucose value. The timing of GV was expressed as time within target range (70-190 mg/dl), time spent in hypoglycemia (blood glucose <70 mg/dl) and time spent in hyperglycemia (blood glucose >191mg/dl) [89,94].

During CGM, participants filled in a 7-day food record for the assessment of their dietary intake.

Table 5 provides the main clinical and metabolic characteristics of participants. The mean postoperative follow-up was 4±2 years (range: 1-7 years). Age, BMI, HbA1c and blood pressure were similar in T2DM remitter and in control subjects. With regard to lipid profile, T2DM remitters showed higher HDL-cholesterol level than control subjects ($p<0.05$) while no difference was observed in the other lipid fractions.

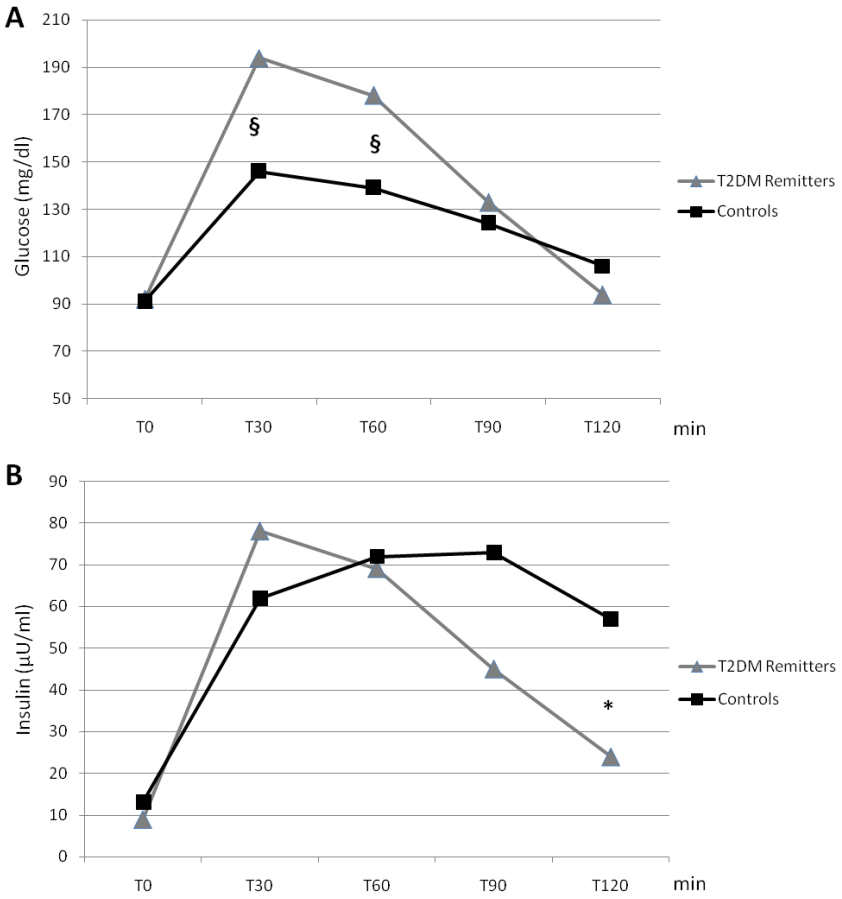
Table 5. Clinical, biochemical and metabolic characteristics of study participants.

	T2DM remitters	**Controls**
Sex (M/F)	10/12	10/12
Age (years)	50±9	52±9
Diabetes duration (years)	4±5	---
RYGB/SG	11/11	---
BMI (Kg/m2) ***Preoperative BMI (Kg/m2)***	31±6 *43±8*	32±6 ---
Time from intervention	4±*2*	---
Fasting blood glucose (mg/dl)	92±17	92±10
HbA_{1c} (%) **HbA_{1c} (mmol/mol)**	5.7±0.6 39±6	5.2±0.3 34±4
Total cholesterol (mg/dl)	187±40	188±44
HDL-cholesterol (mg/dl)	62±16	43±15*
LDL-cholesterol (mg/dl)	105±35	110±30
Triglycerides (mg/dl)	99±43	119±57
Systolic blood pressure (mmHg)	126±8	121±10
Diastolic blood pressure (mmHg)	75±6	72±9

M±SD *p value <0.001

According to general linear model for repeated measures, glucose response during OGTT was significantly higher in T2DM remitter than in control subjects ($p<0.001$). In particular, the difference in plasma glucose level was evident at 30 and 60 min of the test ($p=0.01$, for both) (Figure 8). Similarly, insulin response was significantly different in the two groups ($p<0.001$). In particular, plasma insulin level was significantly lower in T2DM remitter that in control subjects at 120 min ($p<0.05$). Three T2DM remitters showed impaired glucose tolerance (2-h blood glucose between 140 and 199 mg/dl) while all control subjects showed normal glucose tolerance.

Figure 8. Plasma glucose (A) and insulin (B) response to OGTT in T2DM remitter and in control subjects. *= p <0.05; §= p <0.001.



Mean interstitial glucose concentration (IG) throughout the 7-day CGM was similar in the two groups (Table 6); however, T2DM remitters showed higher mean IG peak and lower mean IG nadir than in control subjects (p<0.001 and p=0.03). All measures assessing the amplitude of GV, i.e., SD, CV and MAGE, were significantly higher in T2DM remitters than in control subjects, (p<0.001 for all). The time spent in hyperglycemia (blood glucose >191 mg/dl) as well that spent in hypoglycemia (blood glucose <70 mg/dl), expressed as percentage of the whole monitoring time, were significantly longer in T2DM remitter compared to control subjects (hyperglycemia: 5.0 ± 6.3 vs 0.3 ± 0.5%, p=0.002 and hypoglycemia: 16 ± 20 vs. 2.7 ± 2.9, p=0.007).

Noteworthy, GV was greater in patients operated of RYGB than in those operated of SG, as evidenced by the values of CV (35 ± 5 and 26 ± 4 %, $p<0.05$) and MAGE (136 ± 46 and 99 ± 27 mg/dl, $p<0.05$) in the RYGB and SG group, respectively.

Table 6. Indexes of glucose variability obtained through 7-day CGM and 8-isoprostaglandin F2α (8-isoPGF2α) in DM2 remitter and in control subjects.

	T2DM Remitters	**Controls**	**p value**
Mean IG (mg/dl)	115±23	105±5	0.203
Mean IG peak (mg/dl)	264±58	178±22	< 0.001
Mean IG nadir (mg/dl)	55±16	64±6	0.033
SD (mg/dl)	35±10	21±5	< 0.001
CV (%)	31±6	20±5	< 0.001
MAGE (mg/dl)	118±41	61±19	< 0.001
Time spent at glycemia<70 mg/dl (%)	16±20	2.7±2.9	0.007
Time spent at glycemia 71-191 mg/dl (%)	79±19	97±3	0.007
Time spent at glycemia>191 mg/dl (%)	5±6	0.3±0.5	0.002
8-iso PGF2α (ng/24 h)	1890±1014	1306±459	0.040

M±SD, IG= interstitial glucose, SD= standard deviation, CV= coefficient of variation, MAGE= mean amplitude of glucose excursions

Mean 24-h urinary 8-iso PGF2α excretion was found to be significantly higher in T2DM remitters than that in control subjects (1890 ± 1014 and 1306 ± 459 ng/24h, respectively, $p=0.04$).

All GV indexes were significantly associated with blood glucose levels at 30 min ($p<0.05$-0.001) and SD and MAGE were associated with blood glucose at 60 min during OGTT ($p<0.05$-0.001). No correlation was found between GV indexes and 24-h urinary 8-isoPGF2 α.

Daily caloric intake was similar in the two groups (1550 ± 500 and 1622 ± 434 Kcal /die). With regard to macronutrient distribution, a similar intake of total carbohydrate (48 ± 7 vs. 47 ± 6 %), fat (32 ± 6 vs 34 ± 5 %) and protein (16 ± 3%

and 18 ± 4%) was observed in T2DM remitter compared to control subjects. Likewise, dietary glycemic index and glycemic load were similar in the two groups (59 ± 5 and 117 ± 45 in T2DM remitter and 56 ± 4 and 106 ± 33 in control subjects).

In the present study, we evaluated the daily glucose profile under "real life" conditions in patients in T2DM remission after bariatric surgery. Despite all of them presented values of fasting blood glucose and HbA1c within the normal range, their GV was significantly higher compared with that of age- and BMI-matched control subjects. Quite strikingly, these patients showed an increased oxidative stress, as documented by high urinary excretion of 8-isoPGF2α, indicating that high GV may exert a deleterious effect on the vascular system.

There are consistent data from pathophysiological and clinical studies that high GV may be involved in the pathogenesis of diabetic vascular complications via activation of inflammatory pathways, increased oxidative stress and endothelial dysfunction [95,93,96]. In addition, GV is reported to impact depression, quality of life and other mental health outcomes in diabetic individuals [90]. For these reasons, GV is now considered a therapeutic target in addition to HbA1c, and several studies have focused on the effects of different pharmacologic treatments on GV [97,98]. Although our patients fulfilled the agreed criteria of diabetes remission, they presented an increased GV. Actually, all measures of amplitude of GV were significantly higher than those of control subjects and, more importantly, a considerable amount of time (16% of the monitoring period) was spent in the hypoglycemic range. Previous studies have reported an increased GV in patients undergoing bariatric surgery particularly after RYGB [99,100]. To the best of our knowledge, the present study is the first one to demonstrate that in

addition to high GV, T2DM remitters present an increased oxidative stress, indicating a high risk of vascular damage. The present findings bring us to reconsider the concept of remission of diabetes after bariatric surgery and the criteria for its definition. It is quite clear that the achievement of normal values of fasting blood glucose and HbA1c does not correspond to a true normalization of glucose homeostasis, raising the question whether the term "remission" is appropriate. Furthermore, it could be appropriate to include measures of GV in the assessment of glucose control in patients undergoing bariatric surgery [(101)].

The mechanisms underlying the high GV in our T2DM remitters are not clear. A major source of GV is the rapid onset of postprandial hyperglycemia - as a consequence of accelerated delivery of dietary carbohydrates- followed by reactive hypoglycemia. This mechanism is confirmed by the early and rapid rise in plasma glucose observed during the OGTT. Noteworthy, more than 50% of the exposure to blood glucose <70 mg/dl occurred in the late postprandial period (>3 hours after meals). The relation between postprandial hyperglycemia and GV is further supported by the significant association between 30 and 60 min OGTT blood glucose levels and the amplitude of GV.

It is known that dietary habits with reference to the consumption of high-index glycemic food may contribute to GV both in non-diabetic and diabetic individuals [(102,103)]. The dietary carbohydrate intake of our patients was in the normal range as well as the food glycemic index/glycemic load [(103)]. Thus, it is conceivable that the high GV of our patients is not due to dietary high glycemic load, rather it is a likely consequence of the anatomical changes caused by surgery and, in essence, a price to pay to achieve long-lasting weight loss. This view is in line with the

observation that GV was higher after RYGB than SG, indicating that the more profound the gastro-intestinal anatomical changes the higher the GV.

In conclusion, the remission of T2DM after bariatric surgery is characterized by high GV and increased oxidative stress in the face of fasting blood glucose and HbA1c within the normal range. This finding calls into question the need of critically re-examine the concept of diabetes remission. Long-term studies are needed to assess the impact of these glucose abnormalities on chronic diabetic complications. In the meanwhile, nutritional and/or pharmacologic strategies should be implemented to minimize GV in these patients.

4.1.3 Haemostatic and fibrinolytic parameters

Evidence so far exposed demonstrates that bariatric surgery is able to impact on important metabolic aspects (weight, glucose and lipid homeostasis). On the other hand, all these variables have a strong effect on cardiovascular risk and coagulation system. 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 known to play a relevant role in cardiovascular pathophysiology [(104)]. Several studies suggest that obesity is characterised by an increased expression of several prothrombotic factors, impaired fibrinolysis and platelet hyper-reactivity [(105)]. Weight loss has been found to (partially) revert both metabolic and vascular alterations found in obese subjects [(106)]. However, little is known about the effects of different bariatric surgery techniques on haemostatic and fibrinolytic parameters. We have prospectively evaluated changes in major haemostatic (fibrinogen, D-dimer, coagulation factors II, VII, VIII, IX, X and von

Willebrand factor [vWF], protein C, protein S and antithrombin) and fibrinolytic (PAI-1, t-PA, PAI-1/t-PA ratio) variables in obese subjects undergoing single anastomosis gastric bypass (SAGB) or SG [107]. Consecutive obese subjects referred to the Federico II University Hospital with an indication (according to the European Association for the Study of Obesity guidelines) [108] for bariatric surgery were enrolled in this study. 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. All clinical and laboratory evaluations were repeated in all subjects 60 days (±10 days) after surgery (T1).

A total of 156 obese patients were enrolled in this study, 77 of whom underwent SAGB and 79 who underwent SG. Pre-operatively, the two groups were entirely comparable for all major clinical and demographic characteristics and for all haemostatic and fibrinolytic variables (Table 7). An increase in at least one of haemostatic or fibrinolytic parameters above higher normal levels - suggesting a hypercoagulable state - was found in 37 (48.1%) SAGB and 40 (50.6%) SG patients (p=0.752). At the 2-month post-operative follow-up (T1), a 21.3% reduction in BMI was found in the SAGB group as compared with a 19.1% reduction in the SG group (p=0.139).

Table 7. Clinical and demographic characteristics of the study population before surgery.

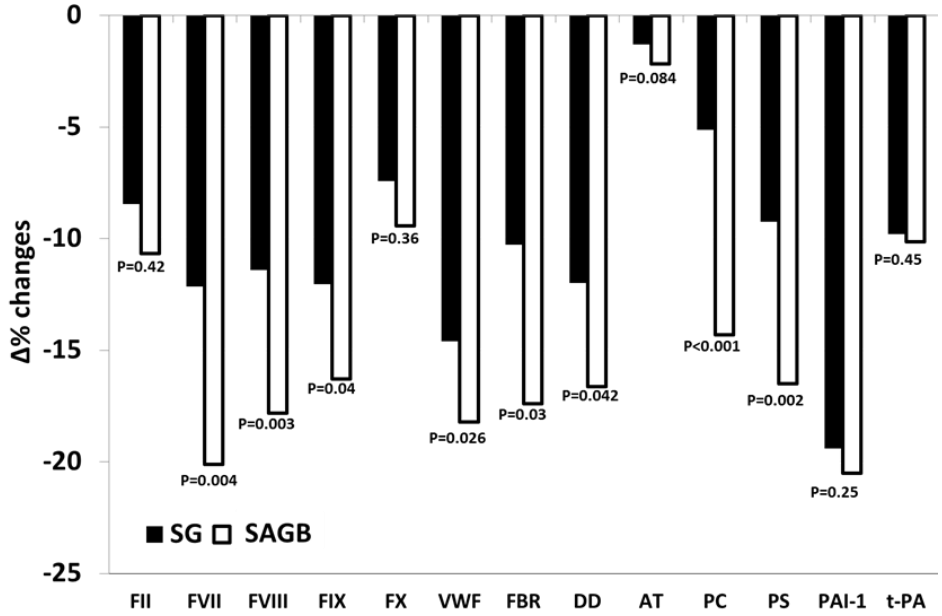
Variable	SG N=79	SAGB N=77	p
Age (years)	37.9±3.1	38.7±3.9	0.186
Male gender	36(45.6%)	45(58.4%)	0.113
BMI	45.3±3.1	44.2±4.7	0.114
PAI-1 antigen	31.7±9.7	33.6±14.5	0.064
t-PA levels	4.8±1.8	5.4±2.0	0.061
Fibrinogen	315.8±48.2	314.1±81.9	0.867
D-Dimer	228.8±232.3	211.9±121.3	0.573
FII	113.7±17.9	117.5±19.1	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
Prot C	116.7±18.3	118.8±15.4	0.441
Prot S	107.4±18.4	113.1±21.0	0.069
AT	97.9±6.2	99.7±7.15	0.096
Hypercholesterolemia	47(59.5%)	38(49.4%)	0.206
Hypertriglyceridemia	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

In parallel, a reduction in haemostatic and fibrinolytic parameters was recorded in both the SAGB and SG groups (Figure 9).

In detail, Δ% changes in the levels of FVII, FVIII, FIX, vWF, fibrinogen, and D-dimer were significantly greater in the SAGB group than in SG group. In addition, SAGB patients showed greater changes in protein C and protein S levels, as compared with those undergoing SG. In contrast, no difference was 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), SAGB 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 9. Δ% changes of haemostatic and fibrinolytic variables 2 months after sleeve gastrectomy (SG) or gastric bypass (SAGB).



Interestingly, at the T1 assessment, 31 subjects (25.9% of SAGB and 13.9% of SG patients, p=0.044) had FVII levels below lower normal cut-off values (i.e., a FVII deficiency), whereas no deficiency of any other clotting factor was found. In these 31 subjects with FVII deficiency, the mean FVII level was 54.5% ± 8.44% (normal reference 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 SAGB patients vs 11.4% of SG patients, p=0.033) and protein S deficiency in 39 (37.6% of SAGB 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 (normal reference range, 50-65%) and the mean level of protein S was 63.1% ±3.79

(normal reference range, 58-69%). Separate multivariate analyses confirmed that GB 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).

These results provide evidence about the effects of bariatric surgery on haemostatic and fibrinolytic balance. We documented that bariatric surgery is able to reduce the hypercoagulable state typical of obese subjects. In detail, we have documented a correlation between post-surgical BMI reduction and changes in PAI-1, with an approximately 20% reduction in PAI-1, one of the major determinants of fibrinolytic potential. Although both SG and SAGB showed a clear efficacy in reducing levels of clotting factors, SAGB was associated with a greater reduction in FVII, protein C and protein S as compared with those following SG. 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 energydependent process [(109)]. The risk of malabsorption and vitamin K deficiency after bariatric surgery is significant, being reported to occur in 20% of patients after malabsorpive procedures [(110)]. Indeed, most of the malabsorptive bariatric procedures (such as SAGB) 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 [(111)]. Although vitamin K-deficiency related changes in clotting factors have been thought to increase the risk of bleeding in patients who undergo bariatric surgery [(112)], data derived from

studies on major inherited bleeding disorders (haemophilia A and B, FVII deficiency) [113,114] clearly indicate that mild deficiencies (clotting factor levels ~40%) in FVII, FVIII and FIX are not associated with an increased risk of bleeding. 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 [115]. 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 [116,117].

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 synthesized through vitamin K-dependent mechanisms, while the production of antithrombin is totally independent of this compound.

In conclusion, we found that bariatric surgery is able to revert the hypercoagulable state usually reported in obese patients; however, in some cases, an acquired natural anticoagulant deficiency may occur and this could be associated with an increased risk of thrombosis [118]. Thus, changes in vitamin status and in haemostatic variables should be strictly monitored after bariatric surgery, particularly when malabsorptive procedures are used.

4.1.4. Surrogate markers of atherosclerosis

Obesity can exert direct negative effects on the atherosclerotic process, as well as on endothelial function [119,120] which are established substrates for cardiovascular disease and strong predictors of future cardiovascular events [121,122].

Carotid intima-media thickness (IMT), flow-mediated dilation (FMD) and nitrate-mediated dilation (NMD) are considered surrogate markers of subclinical atherosclerosis [123,124]. They are widely recognized as independent predictors of CV events [125,126,127,128], thus providing important prognostic data beyond traditional CV risk factors.

Whereas several studies confirmed a significantly increased IMT and impaired FMD and NMD in obese subjects [119,120], few data are available on the effects of bariatric surgery on these markers of CV risk. Thus, we performed a systematic review and meta-analysis of studies evaluating changes in IMT, FMD and NMD in obese patients undergoing bariatric surgery [129].

A total of 10 articles (314 obese patients) were included in the analysis, 6 studies with data on IMT (7 data-sets on 206 patients), 8 studies on FMD (9 data-sets on 269 patients), and 4 on NMD (4 data-sets on 149 patients). All included studies had a prospective design and major characteristics of study populations are shown in Table 8 and Table 9.

Table 8. Characteristics of included studies.

Author	Population (n)	Follow-up (months)	Type of surgery	Reported outcomes	Age (years)	Male gender (%)
Gokce 2005	24	≥ 3	RYGB	FMD	44±10	37
Lind 2009	19	12	RYGB	FMD	41±11	26
Sturm 2009	37	18	RYGB, LAGB	IMT, FMD, NMD	35 (21-52)*	NR
Sarmento 2009	18	12	RYGB	IMT	44,1±9,8	0
Brethauer 2011	15	12	RYGB	FMD, NMD	49,2±10,4	36
Habib (a) 2011	22	6	RYGB	IMT, FMD	44,5±2,4	NR
Habib (b) 2011	28	24	RYGB	IMT, FMD	44,8±1,8	NR
Saleh 2012	47	10	RYGB	IMT, FMD, NMD	41	8,5
Nerla 2012	50	3	RYGB, BPD	FMD, NMD	38±9	26
García 2013	27	12	RYGB, SG	IMT	43,5±8,8	22,5
Tschoner 2013	27	18	RYGB, LAGB	IMT, FMD	33,7 (20-51)*	33

*median value (range)

RYGB: Roux-en-Y Gastric Bypass; LAGB: laparoscopic adjustable gastric banding; BPD: Biliopancreatic Diversion; SG: Sleeve gastrectomy; IMT: Intima-media thickness; FMD: Flow-Mediated Dilation; NMD: Nitrate-Mediated Dilation; NR: Not reported

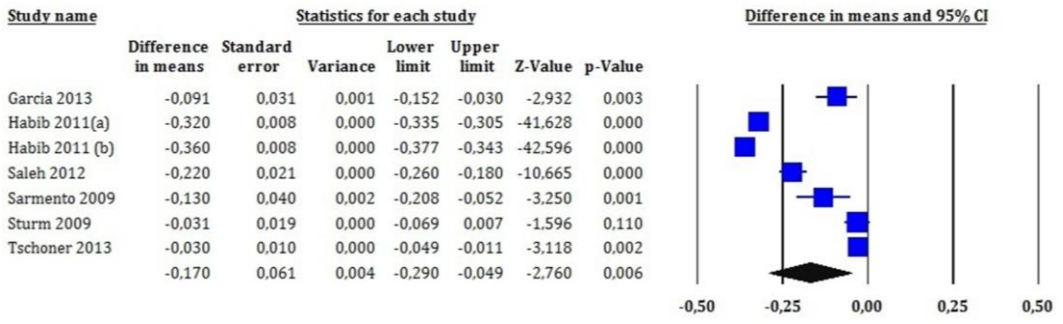
Table 9. Cardiovascular risk factors in included studies.

Author	BMI (Kg/m^2)	Waist (cm)	DM (%)	FPG (mg/dl)	HT (%)	HL (%)	TC (mg/dl)	LDL-c (mg/dl)	HDL-c (mg/dl)	TGs (mg/dl)	Smoking (%)
Gokce 2005	50±8	NR	29	119±39	50	50	NR	NR	NR	NR	8
Lind 2009	43,8±3,1	NR	NR	117±40	NR	NR	NR	124±31	43±8	159±62	NR
Sturm 2009	42,4±3,9	113,7±12,7	NR	98,2±11,5	NR	NR	194,5±40,8	120,1±34,9	49,4±10,2	120 (58)*	14,2
Sarmento 2009	44,3±6,4	120,2±12,8	16,6	97,9±29,6	47	NR	NR	108,5±33,6	51,9±15,7	145,7±72,7	NR
Brethauer 2011	48,7±5,8	132,9±12,5	20	106,7±43,1	73,3	80	200,2±47,7	124,5±38,3	46,4±17,6	146,1±64,5	NR
Habib (a) 2011	47,0±1,0	NR	NR	NR	NR	NR	188±6	105±5	49±1	170±13	NR
Habib (b) 2011	47,2±1,4	NR	NR	NR	NR	NR	188±8	101±7	52±2	169±16	NR
Saleh 2012	47,1±5,5	129±11,2	NR	94,7±21,7	61,7	23,9	183,4±37,7	113,9±29,2	40,4±9,7	148,6±94,6	4,3
Nerla 2012	47,1±8,4	133±17	8	102±23	34	18	196±35	NR	NR	147±30	42
García 2013	38,4±5,0	NR	26	103,1±17,9	68	83	225±50,6	135±35	45±9,3	224±137	26
Tschoner 2013	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR

BMI: body mass index; DM: diabetes mellitus; FPG: fasting plasma glucose; HT: hypertension; HL: hyperlipidemia; TC: Total Cholesterol; LDLc: LDL-cholesterol; HDLc: HDL-cholesterol; TGs: triglycerides; NR: not reported. * Median value (interquartile range).

Six studies [130,131,132,133,134,135] included obese subjects who had undergone RYGB, four studies included patient who had undergone different types of surgery (2 RYGB and LAGB [136,137]; 1 RYGB and BPD [138]; 1 RYGB and SG [139]. In 6 studies (7 data-sets) [132,133,135,136,137,139], obese patients (n=206) showed a significant reduction of IMT (MD: -0.17 mm; 95%CI: -0.290, -0.049; P=0.006) after bariatric surgery (Figure 10). Heterogeneity among these studies was statistically significant (I^2=99.3%; P<0.00001) and no reduction in the overall heterogeneity was found after excluding one study at time. Three studies (4 data-sets) [132,133,135], specifically evaluating a total of 115 subjects who had undergone RYGB, showed an even more relevant IMT reduction (MD: -0.27 mm; 95%CI: -0.33, -0.21; P<0.00001) with a significant heterogeneity among studies (I^2=95.5%; P<0.00001). The significant reduction of IMT after bariatric surgery was confirmed analyzing only the 4 datasets [132,133,135,139] with a short-term follow-up (≤12 month) with a variation of -0.19 (95%CI: -0.303,-0.085; P=0.001 - I^2=96.4%; P<0.00001). In contrast, the 3 datasets [133,136,137] considering a long term follow-up (>12 month) showed no significant variation in IMT after surgery (MD: -0.14 mm; 95%CI: -0.105, 0.387; P=0.263 - I^2=99.7%; P<0.00001).

Figure 10. Changes in common carotid artery intima-media thickness (c-IMT) after bariatric surgery.



Eight studies (9 data-sets) [130,131,133,135,136,137,138,140], evaluating a total of 269 subjects, showed a significant improvement in FMD after bariatric surgery (MD: 5.65%; 95%CI: 2.87, 8.03; P<0.001) (Figure 11). A significant heterogeneity among studies was found (I^2=96.5%; P<0.001), which was not reduced by the exclusion of one study at time. Five studies (6 data-sets) [130,131,133,135,140], evaluating a total of 155 subjects undergoing RYBG, showed an even more relevant FMD improvement (MD: 6.39%; 95%CI: 2.71,10.08; P=0.001) with a significant heterogeneity among studies (I^2=96.1%; P<0.001). Interestingly, changes in FMD were confirmed both in the short-term [131,133, 135,138,140] with a variation of 5.02% (95%CI: 1.92, 8.11; P=0.001 - I^2=94.0%; P<0.001) and in the long-term [133,136,137] with a variation of 6.78% (95%CI: 0.99, 12.57; P=0.022 - I^2=98.6%; P<0.001).

Four studies (4 data-sets) [135,137,138,140] evaluating a total of 149 obese subjects, showed no significant increase of NMD after bariatric surgery (MD: 2.173%; 95%CI: -0.796, 5.142; P=0.151) with a significant heterogeneity among studies (I^2=79.4%; P=0.002). After excluding 2 studies evaluating different surgical procedures [137,138], significantly increased NMD values were found after RYGB (MD: 4.88%; 95%CI: 2.597,7.170; P<0.00001) without heterogeneity among studies (I^2=0%; P=0.370).

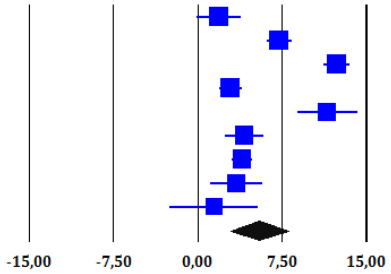
No significant variation in NMD was found either in the only study [137] providing data on >12 months follow-up (MD: 0.410; 95%CI: -3.248;4.068; P=0.826; I^2: not estimable), or in the 3 datasets [135,138,140] considering a ≤12 month follow-up (MD: 2.746; 95%CI: 1.119, 6.690; P=0.172 - I^2=86.2%; P=0.001). However, after excluding the only study with a very short follow-up (3 months) [138], we found a

clear trend toward a significant variation (MD 3.203%; 95%CI: 0.010,6.416; P=0.051) with a marginally significant heterogeneity (I^2=59.5%; P=0.085).

Figure 11. Changes in Flow-mediated dilation (Panel A) and Nitrate-mediated dilation (Panel B) after bariatric surgery.

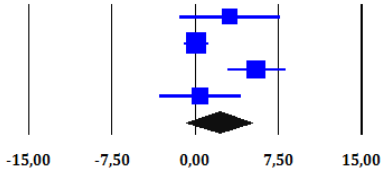
Panel A: Flow-mediated dilation (FMD)

Study name	Difference in means	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Brethauer 2011	1,850	1,018	1,036	-0,145	3,845	1,817	0,069
Habib 2011(a)	7,200	0,576	0,332	6,071	8,329	12,499	0,000
Habib 2011(b)	12,300	0,575	0,330	11,173	13,427	21,400	0,000
Nerla 2012	2,860	0,511	0,261	1,859	3,861	5,598	0,000
Saleh 2012	11,500	1,371	1,881	8,812	14,188	8,385	0,000
Sturm 2009	4,100	0,875	0,766	2,385	5,815	4,685	0,000
Tschoner 2013	3,900	0,462	0,213	2,995	4,805	8,444	0,000
Gokce 2005	3,400	1,155	1,333	1,137	5,663	2,944	0,003
Lind 2009	1,400	1,997	3,988	-2,514	5,314	0,701	0,483
	5,449	1,317	1,733	2,869	8,030	4,139	0,000



Panel B: Nitrate-mediated dilation (NMD)

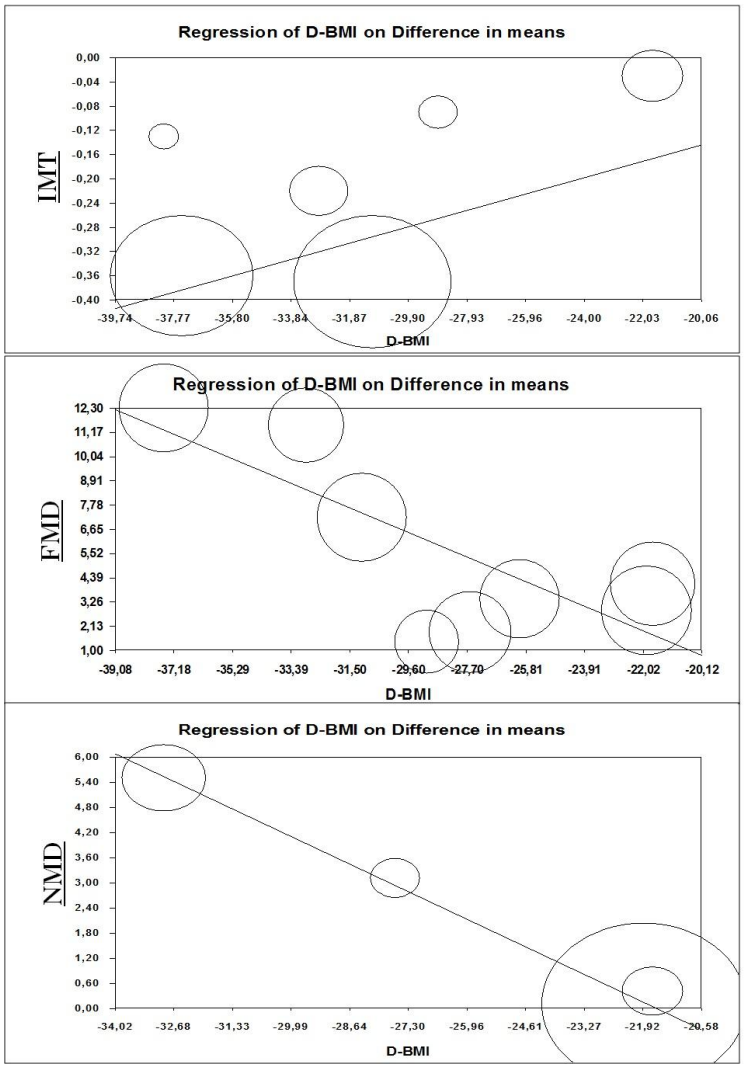
Study name	Difference in means	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Brethauer 2011	3,110	2,297	5,276	-1,392	7,612	1,354	0,176
Nerla 2012	0,100	0,557	0,311	-0,992	1,192	0,179	0,858
Saleh 2012	5,500	1,354	1,834	2,846	8,154	4,061	0,000
Sturm 2009	0,410	1,867	3,484	-3,248	4,068	0,220	0,826
	2,173	1,515	2,295	-0,796	5,142	1,435	0,151



Regression models showed that age, BMI, waist circumference and basal IMT significantly impacted on changes of IMT (Z= -4.55, P<0.001; Z= -3.63, P<0.001; - Z= -6.66, P<0.001; Z= -12.72, P<0.001, respectively) while LDLc levels were inversely associated with the reduction in IMT values (Z= 4.14, P<0.001). Moreover, percentual changes in BMI (Figure 12), body weight, waist circumference, SBP and DBP from baseline to post-operative values were associated with IMT variation (Z= 11.52, P<0.001; Z= 2.79, P=0.005; Z= 2.28, P=0.002; Z= 2.49, P=0.01; Z= 2.51, P=0.01; respectively). In addition, male gender, fasting plasma glucose, total cholesterol and LDL-c significantly impacted on changes in FMD (Z= -3.04, P=0.002; Z= -2.86, P=0.0; Z= -3.61, P<0.001; Z= -3.13, P=0.002, respectively). Percentual changes in BMI (Figure

12) and in body weight significantly correlated with FMD improvement (Z= -4.26, P<0.001; Z= -2.42, P=0.016). Accordingly, fasting plasma glucose, percentual changes in BMI (Figure 12), body weight and waist circumference significantly correlated with NMD improvement (Z=-2.70, P=0.007; Z=-3.81, P<0.001; Z=-3.68, P<0.001; Z=-3.79, P<0.00). Overall, percentual changes in IMT values were significantly associated with changes in FMD (Z=-2.55, P=0.01). All the other co-variates tested did not impact on IMT, FMD and NMD.

Figure 12. Meta-regression analysis. Association of Body Mass Index percentual changes with modifications in carotid intima-media thickness, flow-mediated dilation and nitrate-mediated dilation.



BMI: Body Mass Index; IMT: Intima-media thickness; FMD: Flow-Mediated Dilation; NMD: Nitrate-Mediated Dilation; D: percentual changes from baseline values to post-operative values

The results of the present meta-analysis on 314 obese patients show that bariatric surgery is associated with a significant improvement of structural and functional markers of atherosclerosis. In detail, subjects undergoing bariatric surgery showed a significant reduction of carotid IMT (-0.17 mm), accompanied by a 5.6% increase in FMD. In line with these findings, NMD showed a tendency toward improvement after bariatric surgery, reaching the statistical significance only in studies evaluating RYGB.

Meta-regression analyses showed that the older age, the higher body weight and waist circumference, the higher the IMT. In contrast, the lower the LDL-c levels at baseline the larger was the reduction of IMT after bariatric surgery. Moreover, whereas the male gender, fasting plasma glucose levels, total cholesterol and LDL-c predicted higher changes in FMD, variations in NMD were predicted only by fasting plasma glucose levels. As a further confirmation, we also found that changes in weight, BMI, waist circumference, systolic and diastolic blood pressure significantly and positively predicted the modifications of IMT. Interestingly, changes in body weight, BMI and waist circumference resulted associated with changes in FMD and/or NMD.

Overall, our data suggest a consistent cardioprotective effect of bariatric surgery through its beneficial effects on subclinical atherosclerosis and on endothelial function.

Several mechanisms may be involved in the improvement of these parameters after bariatric surgery. In detail, bariatric surgery is effective in reducing the main cardiovascular risk factors such as insulin resistance, type 2 diabetes, hypertension, hyperlipidemia and that the magnitude of this reduction exceeds the effect of weight loss itself [51,141]. Moreover, bariatric surgery exerts an important

modulation on the haemostatic and fibrinolytic balance being able to reduce the hypercoagulable state typical of obese subjects [107]. It is important to highlight that many of these effects of bariatric surgery are observed within the first 12 month of the postoperative period. Accordingly, we demonstrated that the improvement of IMT and FMD is relevant at the short term follow-up (≤12 months), while it becomes less evident thereafter.

In conclusion, in our meta-analysis bariatric surgery is associated with an improvement of subclinical atherosclerosis and endothelial function. These effects may significantly contribute to the reduction of cardiovascular risk in patients who undergo bariatric operations. This evidence further supports the use of bariatric surgery as a lifesaving therapy with a goal of reducing cardiovascular morbidity/mortality, especially in patients with a high obesity-related cardiovascular risk.

4.2 Bariatric surgery and nutritional issues

Beside surgical complications, one of the major concerns of bariatric surgery is the worsening of nutritional status. Indeed, nutritional deficiencies are well-recognized complications after different bariatric procedures.

Several studies have assessed the nutritional status after bariatric surgery, reporting multiple deficiencies especially after malabsorptive procedures, such as RYGB and BPD. Of note, these nutritional complications have been described also after restrictive interventions such as SG and AGB.

There are several reports on micronutrient deficiencies, (i.e., vitamins A, D, B1, B6, folic acid) and minerals (calcium, iron, zinc, copper and magnesium). Macronutrient deficiencies or clinical events related to frank malnutrition (total energy intake less than 50% the nutritional needs [142,143] are less frequent [144]. Osteomalacia/osteoporosis and anemia are the most widely reported adverse clinical outcomes [145,146]. Protein malnutrition and anemia, among others, could be attributed to 1) reduced food intake (vomiting, food aversion, modified eating behavior and nonadherence to dietary recommendations), 2) reduced gastric acid secretion, and/or malabsorption following the exclusion of sites of absorption of various minerals and vitamins (duodenum and proximal jejunum) [142].

A growing literature now attests the occurrence of nutrient deficiencies in morbidly obese individuals prior to bariatric surgery, which may be aggravated by surgical procedures, resulting in more serious postoperative complications. Thus, we performed a systematic review and meta-analysis to evaluate the prevalence of nutrient deficiencies in obese patients before bariatric surgery.

A total of 29 articles (32 datasets) were included in the analysis. The major characteristics of the included studies are shown in Table 10.

Table 10. Major characteristics of included studies.

Author	Design	Procedure	Reported outcomes
Ben-porat 2015 [147]	Retrospective	SG	Hb, Iron, Ferritin, Folate, Vit B12, Vit D
Blume 2012 [148]	Retrospective	RYGB	Albumin, Hb, Iron, Ferritin, Folate, Vit B12
Carrodeguas 2005 [149]	Retrospective	AGB, RYGB	Vit B1
Censani 2013 [150]	NR	Restrictive	Vit D
Dagan 2016 [151]	NR	SG	Hb, Iron, Ferritin, Folate, Vit B1, Vit B12, Vit D
Damms-Machado 2012 [152]	NR	SG	Ca, K, Iron, Folate, Vit B1, Vit B6, Vit B12, Vit A, Vit E, Vit D
de Luis 2013 [153]	NR	BPD	Albumin, Cu, Zn, Ca, P, Hb, Ferritin, Folate, Vit B12, Vit A, Vit E, Vit K, Vit D
Ernst 2009 [154]	NR	NR	Albumin, Zn, Mg, P, Hb, Ferritin, Folate, Vit B12, Vit D
Ewang-Emukowhate 2015 [155]	Prospective	NR	Iron, Folate, Vit B12, Vit K, Vit D
Flancbaum 2006 [156]	Retrospective	RYGB	Albumin, Ca, Hb, Iron, Ferritin, Vit B1, Vit B12, Vit D
Gehrer 2010 [157]	Prospective	SG, RYGB	Albumin, Zn, Ca, Iron, Folate, Vit B1, Vit B6, Vit B12, Vit D
Gemmel 2009 [158]	Retrospective	AGB, SG, RYGB, BPD	Folate, Vit B12, Vit A, Vit D
Gerig 2013 [159]	Retrospective	NR	Cu, Zn, Mg, Ca, P, Hb, Ferritin, Folate, Vit B12, Vit D
Gillon 2016 [160]	NR	SG	Hb, Ferritin, Folate, Vit B12, Vit D
Gobato 2014 [161]	Prospective	RYGB	Cu, Zn, Mg, Iron, Ferritin, Folate
Jin 2009 [162]	Retrospective	RYGB	Vit D
Lefebvre 2014 [163]	Crossectional	NR	Zn, Se, Mg, Ca, P, Hb, Iron, Ferritin, Vit B12, Vit A, Vit D
Madan 2006 [164]	Retrospective	RYGB	Zn, Se, Iron, Ferritin, Folate, Vit B12, Vit A, Vit D
Moize 2011 [165]	Retrospective	NR	Albumin, Zn, Mg, Ca, Hb, Iron, Ferritin, Folate, Vit B1, Vit B6, Vit B12, Vit D
Papamargaritis 2015 [166]	Retrospective	AGB, SG, RYBG	Cu, Zn, Se
Peterson 2016 [167]	Prospective	RYGB	Iron, Folate, Vit B1, Vit B12, Vit A, Vit E, Vit D
Sanchez 2016 [168]	NR	SG, RYGB	Albumin, Cu, Zn Ca, P, Hb, Iron, Ferritin, Folate, Vit B12, Vit D
Schiavo 2016 [169]	Prospective	NR	Zn, Se, Mg, Iron, Folate, Vit B12, Vit A, Vit C, Vit E, Vit D
Schweiger 2010 [170]	NR	AGB, SG, RYGB, BPD	Albumin, Ca, P, Hb, Iron, Ferritin, Folate, Vit B12
Skroubis 2002 [171]	Retrospective	RYGB, BPD	Hb, Iron, Ferritin, Vit B12
Toh 2009 [172]	Retrospective	AGB, SG, RYGB	Albumin, Hb, Iron, Ferritin, Folate, Vit B12, Vit D
van Rutte 2014 [173]	Retrospective	SG	Albumin, Zn, Mg, Ca, P, Hb, Iron, Ferritin, Folate, Vit B1, Vit B6, Vit B12, Vit A, Vit D
Wang 2016 [174]	Retrospective	NR	Albumin, Mg, Ca, P, K, Iron, Ferritin, Folate, Vit B12, Vit D
Wolf 2015 [175]	NR	SG	Albumin, Mg, Ca, P, Vit A, Vit C, Vit D

RYGB: Roux-en-Y Gastric Bypass; AGB: adjustable gastric banding; BPD: Biliopancreatic Diversion; SG: Sleeve gastrectomy; NR: not reported; Ca: calcium; Cu: copper; Hb: hemoglobin; K: potassium; Mg: magnesium; P: phosphate; Se: selenium; Vit: vitamin; Zn: zinc.

Fourteen studies had a retrospective design, six were prospective while for the remainders the design was not clearly reported. Moreover, a range of micronutrient and vitamin deficiencies were assessed in the different studies.

The outcomes included: albumin, copper, zinc, magnesium, potassium, calcium, phosphate, hemoglobin, iron, ferritin, folate, vitamin B1, B6, B12, A, C, E, K, 25 (OH) vitamin D. As reported in Table 11, 25 (OH) vitamin D deficiency (i.e. 25 (OH) vitamin D < 20 ng/dl) was the most frequent deficit in the studies analyzed (59.8%, 95%CI: 51.1-67.9) with a prevalence of severe deficiency (i.e. 25 (OH) vitamin D < 10 ng/dl) of 19.2% (95%CI: 14.4-25.1). Similarly, we found a high prevalence of deficit of vitamin C, iron and zinc (23.4%, 21.7% and 14.8%, respectively).

Table 11. Weighted mean prevalence (WMP) of micronutrient and vitamin deficiencies in obese patients before bariatric surgery.

Outcome	Datasets (n)	Subjects (n)	WMP (%)	95%CI	I^2	P
Albumin	12	2288	4	1.6-9.8	93.3	< 0.001
Hemoglobin	19	4046	8.6	6.2-11.8	89.9	< 0.001
Iron	20	2564	21.7	16.0-28.7	92.7	< 0.001
Ferritin	20	3778	6.2	4.6-8.3	77.2	< 0.001
Folate	21	3178	7.6	4.8-11.8	91.8	< 0.001
Vitamin B1	7	1105	8.4	4.1-16.6	91.2	< 0.001
Vitamin B6	4	429	5.4	1.6-16.9	81.7	0.001
Vitamin B12	24	4045	7.4	5.2-10.3	88.7	< 0.001
Vitamin A	9	1096	6.5	3.6-11.4	73.2	< 0.001
Vitamin C	2	83	23.4	10.3-44.9	70.2	0.067
Vitamin D <30 ng/dl	12	1597	87.8	72.9-95.1	97.2	< 0.001
Vitamin D <20 ng/dl	17	2248	59.8	51.1-67.9	93.0	< 0.001
Vitamin D <10 ng/dl	7	1354	19.2	14.4-25.1	82.2	< 0.001
Vitamin E	4	267	2.1	0.6-6.8	24	0.267
Vitamin K	2	233	6.1	0-89.9	91.9	< 0.001
Zinc	12	1623	14.8	7.4-27.5	95.1	< 0.001
Copper	5	702	4.7	0.4-39.8	97.7	< 0.001
Magnesium	9	1574	4	1.4-10.6	94.7	< 0.001
Potassium	2	241	5.8	3.5-9.6	0	0.830
Selenium	4	365	11.2	1.5-50.3	95.9	< 0.001
Calcium	12	2160	2.2	1.0-4.8	84.7	< 0.001
Phosphate	9	1615	7.6	4.6-12.3	85.3	< 0.001

Although nutritional deficiencies in obesity are multifactorial, low quality diet may greatly contribute to these events. Indeed, high intake of high caloric density foods with low nutritional quality may not be able to meet the individual micronutrient needs. In addition, other mechanisms may be involved, such as defective storage and bioavailability of some nutrients (e.g., vitamin D), increased hepcidin synthesis leading to reduced iron absorption due to chronic inflammation, and overgrowth of small intestinal bacterial, which may consume vitamin B1 and B12 and fat soluble vitamins leading to their shortage [176].

Vitamin D deficiency deserves a particular comment. Beside its consequences on several body organ systems [177,178,179], vitamin D deficiency can cause unbalanced resorptive bone loss leading to osteopenia and osteoporosis. This is particularly important in the bariatric population because these patients are at risk for fractures, being predominantly females and perimenopausal. Vitamin D insufficiency is a common issue in obese individuals as well as in bariatric patients [180], and obesity *per se* represents a risk factor for vitamin D deficiency and often requires substantial supplementation to achieve sufficiency [181]. It has been hypothesized that obese individuals are more likely to be deficient in vitamin D because of the higher volumetric dilution and sequestration of this fat-soluble hormone in the adipose tissue [182]. As fat mass increases, an individual will require greater amounts of vitamin D (via photoproduction from sun exposure, dietary intake, and/or supplementation). Moreover, although there is no difference in vitamin D_3 production between obese and lean individuals, obese patients may have impaired release of vitamin D_3 from the skin [183]. Genetic variation in the function of the vitamin D binding protein and vitamin D receptor may also

influence 25(OH)D levels, with some studies suggesting higher frequency of the poorer functioning forms in obesity [184,185].

Interestingly, in our study we found a high heterogeneity among studies in most of the evaluated outcomes (Table 11). This could be due to differences in study design and number of studies. Accordingly, we performed some sensitivity analyses including only studies with a prospective design (Table 12). This sensitivity analysis confirmed that the most frequent deficiencies involved 25(OH)vitamin D, iron and zinc.

It is noteworthy that the number of studies reporting the analyzed outcomes was highly variable, ranging from 21 to 2 studies (Figure 13). In particular, for Vitamin B6, Vitamin C, Vitamin E, Vitamin K, Copper, Potassium and Selenium ≤ 5 studies are available. This should be taken into account when interpreting the present results and could suggest that, for some micronutrients and vitamins, further prospective studies are needed to definitely estimate the prevalence of deficiency in obese subjects before bariatric surgery.

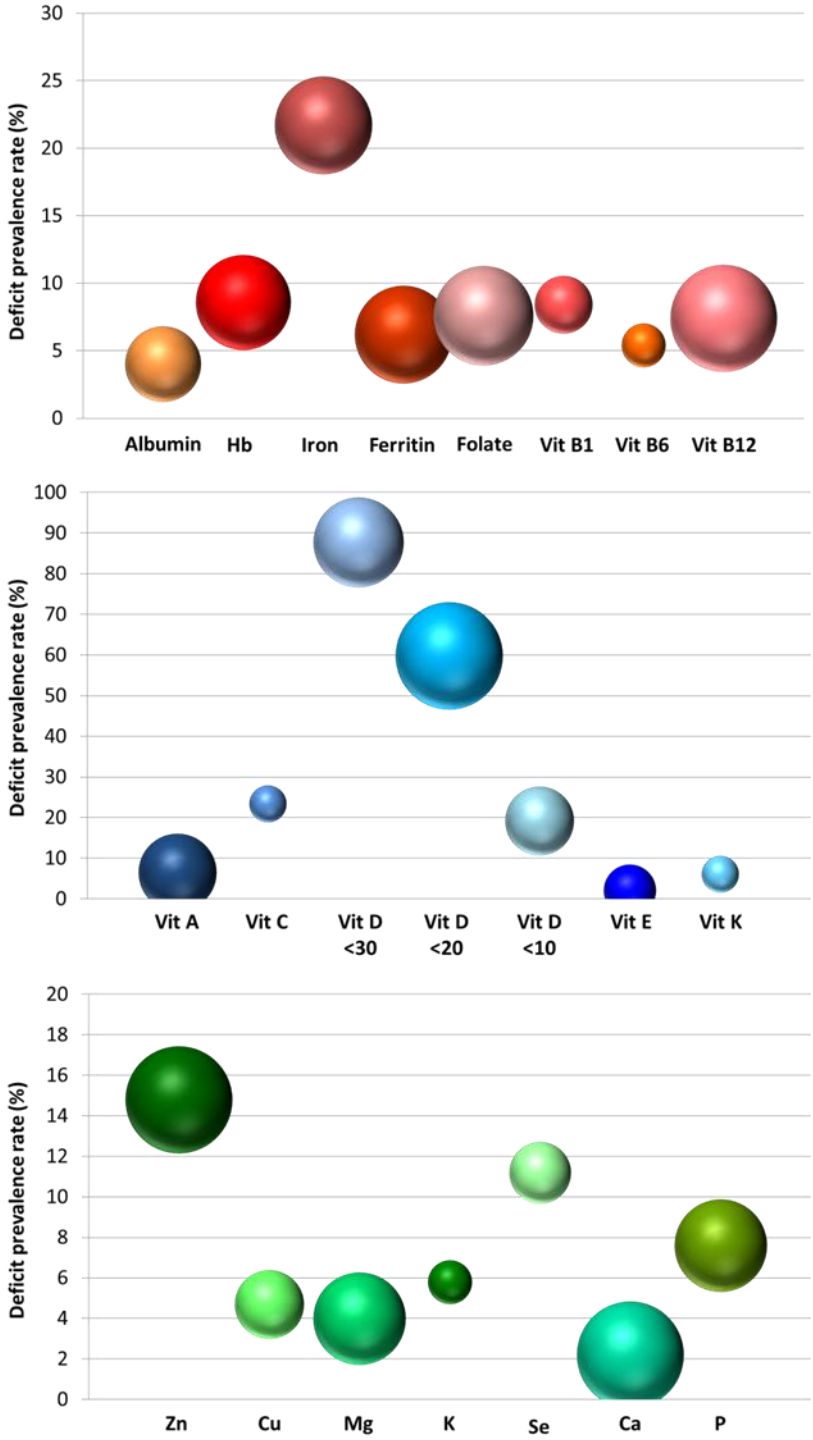
Table 12. Sensitivity analysis including studies with a prospective design.

Outcome	Datasets (n)	Subjects (n)	WMP (%)	95%CI	I^2	P
Albumin	0	-	-	-	-	-
Hemoglobin	1	-	-	-	-	-
Iron	6	614	20.2	9.8-37.2	92.5	<0.001
Ferritin	2	263	5.4	3.2-9.0	0	0.474
Folate	5	388	7.6	3.1-17.2	77.5	0.001
Vitamin B1	2	194	1.0	0.2-4.9	0	0.368
Vitamin B6	1	-	-	-	-	-
Vitamin B12	5*	579	8.3	3.4-18.6	88.1	<0.001
Vitamin A	3**	317	9.6	3.6-23.3	69.8	0.037
Vitamin C	1	-	-	-	-	-
Vitamin D <30 ng/dl	3	216	86.3	63.2-95.8	88.8	<0.001
Vitamin D <20 ng/dl	3	418	53.3	22.8-81.5	97.1	<0.001
Vitamin D <10 ng/dl	2	342	24.6	20.3-29.4	0	0.795
Vitamin E	2	98	4.2	1.5-11.3	0	0.335
Vitamin K	1	-	-	-	-	-
Zinc	4	434	12.7	3.2-39.4	92.6	<0.001
Copper	1	-	-	-	-	-
Magnesium	3	296	6.8	0.5-51	86.8	0.001
Potassium	0	-	-	-	-	-
Selenium	2	262	7.0	1.4-28.1	87.9	0.004
Calcium	2	399	1.1	0.4-3.1	0	0.397
Phosphate	1	-	-	-	-	-

*After exclusion of the study by Ewang et al that included patients receiving nutrients supplementation and used a cut-off to define Vitamin B12 deficiency different from the other we obtain a WMP: 6.1% (95% CI: 3.4-10.7) I^2: 45.3%, p=0.140

** After exclusion of the study by Lefebvre et al that used a cut-off to define Vitamin A deficiency different from the other we obtain a WMP: 5.1%(95%CI: 0.9-24) $I^2$61.9%, p=0.105

Figure 13. Weighted mean prevalence of micronutrient and vitamin deficiencies in obese patients before bariatric surgery.



The size of each circle is proportional to the number of datasets for each outcome.
Ca: calcium; Cu: copper; Hb: hemoglobin; K: potassium; Mg: magnesium; P: phosphate; Se: selenium; Vit: vitamin; Zn: zinc.

5. Conclusions

Extensive evidence supports the efficacy of bariatric surgery in reducing body weight and obesity-related comorbidities in severely obese patients. In our studies, we found a significant improvement of glucose and lipid homeostasis in obese T2DM patients 1-2 years after bariatric surgery. The rate of diabetes remission at 1 year was 76% after SG and 86% after RYGB and the two major determinants of glucose homeostasis, i.e. beta cell function and insulin sensitivity, improved to a similar extent after either procedures. These results were achieved in the face of a different pattern of GI hormone profile, suggesting that weight loss and the consequent improvement of insulin sensitivity are the main determinants of diabetes remission, at least several months after surgery. Interestingly, while plasma triglycerides decreased to a similar extent with the two procedures, total and LDL-cholesterol decreased more consistently after RYGB than SG; furthermore, the decrease in LDL-cholesterol was inversely related to meal-induced GLP-1 response suggesting that GLP-1 restoration is crucial for the improvement of cholesterol metabolism, possibly through an increase in circulating bile acids.

The overall metabolic improvement induced by bariatric surgery translates into clear benefits in terms of cardiovascular risk. Indeed, we have documented a reduction in carotid intima-media thickness and an increase in endothelial function after bariatric procedures. These changes in markers of subclinical atherosclerosis are in line with the reduction in cardiovascular morbidity and mortality, consistently shown by large population studies. Thus, the cardioprotective effects of bariatric surgery range from improvement in early signs of subclinical atherosclerosis to prevention of major fatal and non-fatal

cardiovascular and cerebrovascular events. Further extending this finding, we have documented that bariatric surgery is able to improve the obesity-related hypercoagulable state characterized by increased levels of clotting factors and impaired fibrinolysis. However, it is important to note that at 2-month post-operative follow-up, we observed a significant decrease in natural anticoagulants, probably due to reduction of vitamin K absorption. This decrease could potentially lead to increased thrombotic risk. Since our data have been obtained in the early post-operative phase, no definite conclusion on the long-term effect of bariatric surgery on coagulant/anticoagulant balance can be drawn.

Despite the proven efficacy of bariatric surgery in improving overall metabolic control and reducing total and cardiovascular mortality, some concerns can be raised regarding two main points: 1) increased glucose variability and 2) alterations of nutritional status.

With regard to the former, we have found that some patients classified as diabetes remitters according to currently validated criteria, suffer from high glycemic variability, i.e., they present ample glucose excursions throughout the day, often reaching frank hypoglycemic threshold. This alteration is likely to be underdiagnosed since it can be detected only by continuous glucose monitoring. Even more interesting is the observation that high GV is associated with increased oxidative stress, indicating an increased risk of vascular damage. Based on these findings, we propose that GV be included among criteria for the definition of diabetes remission in order to have a comprehensive picture of the impact of bariatric surgery on glucose homeostasis. Furthermore, since high GV is likely involved in the pathogenesis of vascular complications and mortality risk, at least in diabetic patients, ad hoc studies should be performed to identify and to manage

appropriately glycemic variability. Of great interest is the possibility to reduce GV by proper nutritional measures, such as low-glycemic index food.

The latter issue is one that involves nutrient deficiencies. Several studies have consistently documented a high prevalence of vitamin and mineral deficiencies after bariatric procedures. Poor postoperative nutrient intake, recurrent vomiting, inadequate supplementation are important risk factors. In addition, reviewing the available literature, we have documented that a considerable number of obese patients present vitamin and/or micronutrient deficiencies already before surgery, vitamin D deficit being the most frequent abnormality (about 60%). The high prevalence of pre-operative nutritional deficiency underlines the need of a careful nutritional screening in all patients scheduled for bariatric surgery in order to detect and correct any possible deficiency before intervention. Likewise, effective strategies should be implemented to improve long-term patients' adherence to lifestyle and nutritional recommendations in order to maximize the benefits of bariatric surgery and reducing the risk of the above discussed complications.

References

[1] Finucane MM, Stevens GA, Cowan MJ, Danaei G, Lin JK, Paciorek CJ, Singh GM, Gutierrez HR, Lu Y, Bahalim AN, Farzadfar F, Riley LM, Ezzati M; Global Burden of Metabolic Risk Factors of Chronic Diseases Collaborating Group (Body Mass Index). National, regional, and global trends in bodymass index since 1980: systematic analysis of health examination surveys and epidemiological studies with 960 country-years and 9.1 million participants. Lancet. 2011;377:557–67.

[2] World Health Organization (WHO), available at: http:// www.euro.who.int/en/ what-we-do/health-topics/noncommunicable-diseases/ obesity.

[3] World Health Organization (WHO), Fact Sheet No.311 (updated March 2013) available at: http://www.who.int/mediacentre/ factsheets/fs311/en/.

[4] Frühbeck G, Toplak H, Woodward E, Yumuk V, Maislos M, Oppert JM; Executive Committee of the European Association for the Study of Obesity. Obesity: the gateway to ill health—an EASO Position Statement on a rising public health, clinical and scientific challenge in Europe. Obes Facts. 2013;6:117– 20.

[5] Kelly T, Yang W, Chen CS, et al. Global burden of obesity in 2005 and projections to 2030. Int J Obes (London). 2008;32:1431–7.

[6] Sjöström L. Review of the key results from the Swedish Obese Subjects (SOS) trial—a prospective controlled intervention study of bariatric surgery. J Intern Med. 2013;273:219–34.

[7] Calle EE, Thun MJ, Petrelli JM. Body-mass index and mortality in a prospective cohort of US adults. N Engl J Med 1999;341:1097–1105.

[8] Yoon K, Lee J-H, Kim J-W, Cho JH, Choi Y-H, Ko S-H, Zimmet P, Son HY: Epidemic obesity and type 2 diabetes in Asia. Lancet 2006;368:1681–1686.

[9] Golay A, Ybarra J. Link between obesity and type 2 diabetes. Best Pract Res Clin Endocrinol Metab 2005;19:649-63.

[10] Sjöström L, Lindroos AK, Peltonen M et al. Lifestyle, diabetes, and cardiovascular risk factors 10 years after bariatric surgery. N Engl J Med 2004;351:2683-93.

[11] Sjöström L, Gummesson A, Sjöström CD et al. Effects of bariatric surgery on cancer incidence in obese patients in Sweden (Swedish Obese Subjects Study): a prospective, controlled intervention trial. Lancet Oncol 2009;10:653-62.

[12] Sjöström L, Peltonen M, Jacobson P et al. Bariatric surgery and long-term cardiovascular events. JAMA 2012;307:56-65.

[13] Schauer PR, Kashyap SR, Wolski K et al. Bariatric surgery versus intensive medical therapy in obese patients with diabetes. N Engl J Med 2012;366:1567-76.

[14] Ikramuddin S, Korner J, Lee WJ et al. Roux-en-Y gastric bypass vs intensive medical management for the control of type 2 diabetes, hypertension, and hyperlipidemia: the Diabetes Surgery Study randomized clinical trial. JAMA 2013;309:2240-9.

[15] Christou NV, Lieberman M, Sampalis F, Sampalis JS. Bariatric surgery reduces cancer risk in morbidly obese patients. Surg Obes Relat Dis 2008;4:691-5.

[16] Busetto L, Mirabelli D, Petroni ML et al. Comparative long-term mortality after laparoscopic adjustable gastric banding versus nonsurgical controls. Surg Obes Relat Dis 2007;3:496-502.

[17] Poirier P, Cornier MA, Mazzone T et al. Bariatric surgery and cardiovascular risk factors: a scientific statement from the American Heart Association. Circulation 2011;123:1683-701..

[18] Pontiroli AE, Morabito A. Long-term prevention of mortality in morbid obesity through bariatric surgery. A systematic review and meta-analysis of trials performed with gastric banding and gastric bypass. Ann Surg 2011;253:484-7.

[19] Karlsson J, Taft C, Rydén A et al. Ten-year trends in health-related quality of life after surgical and conventional treatment for severe obesity: the SOS intervention study. Int J Obes 2007;31:1248-61.

[20] Pories WJ, Dohm LG, Mansfield CJ. Beyond the BMI: the search for better guidelines for bariatric surgery. Obesity 2010;18:865-71.

[21] Busetto L, Dixon J, De Luca M et al. Bariatric Surgery in Class I Obesity: a position statement from the International Federation for the Surgery of Obesity and Metabolic Disorders (IFSO). Obes Surg 2014;24: 487-519.

[22] O'Brien PE, Dixon JB, Laurie C et al. Treatment of mild to moderate obesity with laparoscopic adjustable gastric banding or an intensive medical program: a randomized trial. Ann Intern Med 2006;144:625-33.

[23] Dixon JB, O'Brien PE, Playfair J et al. Adjustable gastric banding and conventional therapy for type 2 diabetes: a randomized controlled trial. JAMA 2008;299:316-23.

[24] ASMBS Clinical Issues Committee. Bariatric surgery in class I obesity (BMI 30-35 kg/m2). Surg Obes Relat Dis 2013;9:e1-e10.

[25] Li Q, Chen L, Yang Z et al. Metabolic effects of bariatric surgery in type 2 diabetic patients with body mass index of 35 kg/m2 . Diabetes Obes Metab 2012;14:262-70.

[26] Panunzi S, De Gaetano A, Carnicelli A, Mingrone G. Predictors of remission of diabetes mellitus in severely obese individuals undergoing bariatric surgery: do BMI or procedure choice matter? A meta-analysis. Ann Surg. 2015 Mar;261(3):459-67.

[27] Società Italiana di Chirurgia dell'Obesità e delle malattie metaboliche. Linee guida di chirurgia dell'obesità. Edizione 2016

[28] Barber W, Burke TF. Brain-stem response to phasic gastric distension. Am J Phisiol 1983;245:G242

[29] Chambers AP, Smith EP, Begg DP, Grayson BE, Sisley S, Greer T, Sorrell J, Lemmen L, LaSance K, Woods SC, et al. Regulation of gastric emptying rate and its role in nutrient-induced GLP-1 secretion in rats after vertical sleeve gastrectomy. Am J Physiol Endocrinol Metab. 2014;306:E424–E432.

[30] Trung VN, Yamamoto H, Furukawa A, Yamaguchi T, Murata S, Yoshimura M, Murakami Y, Sato S, Otani H, Ugi S, et al. Enhanced Intestinal Motility during Oral Glucose Tolerance Test after Laparoscopic Sleeve Gastrectomy: Preliminary Results Using Cine Magnetic Resonance Imaging. PLoS One.

[31] David Benaiges, Antonio Más-Lorenzo, Albert Goday, José M Ramon, Juan J Chillarón, Juan Pedro-Botet, Juana A Flores-Le Roux. Laparoscopic sleeve gastrectomy: More than a restrictive bariatric surgery procedure? World J Gastroenterol. 2015 Nov 7; 21(41): 11804–11814.

[32] Scopinaro N, Gianetta E, Civalleri D, Bonalumi U, Bachi V. Biliopancreatic by-pass for obesity. II. Initial experience in man. *British Journal of Surgery*. 1979;66(9):618–620.

[33] Rubino F, Schauer PR, Kaplan LM, Cummings DE. Metabolic surgery to treat type 2 diabetes: clinical outcomes and mechanisms of action. Annu Rev Med. 2010;61:393–411.

[34] Pories WJ. Bariatric surgery: risks and rewards. J Clin Endocrinol Metab. 2008;93:s89–s96.

[35] G Abdeen, CW le Roux. Mechanism Underlying the Weight Loss and Complications of Roux-en-Y Gastric Bypass. Obes Surg. 2016; 26: 410–421.

[36] Wang W, Wei PL, Lee YC, et al. Short-term results of laparoscopic mini-gastric bypass. Obes Surg. 2005;15(5):648–54.

[37] Angrisani L, Santonicola A, Iovino P, Formisano G, Buchwald H, Scopinaro N. Bariatric surgery worldwide 2013. Obes Surg 2015;25:1822– 1832

[38] Khorgami Z, Andalib A, Corcelles R, Aminian A, Brethauer S, Schauer P. Recent national trends in the surgical treatment of obesity: sleeve gastrectomy dominates. Surg Obes Relat Dis 2015;11:S6–S8

[39] Belle SH, Berk PD, Courcoulas AP, et al.; LABS Consortium Weight Change Writing Group. Reporting weight change: standardized reporting accounting for baseline weight. Surg Obes Relat Dis 2013;9:782–789

[40] Buchwald H, Avidor Y, Braunwald E, et al. Bariatric surgery: a systematic review and metaanalysis. JAMA 2004;292:1724–1737

[41] Brethauer SA, Hammel JP, Schauer PR. Systematic review of sleeve gastrectomy as staging and primary bariatric procedure. Surg Obes Relat Dis 2009;5:469–475

[42] Wing RR, Bolin P, Brancati FL, et al.; Look AHEAD Research Group. Cardiovascular effects of intensive lifestyle intervention in type 2 diabetes. N Engl J Med 2013;369:145–154

[43] Schauer PR, Mingrone G, Ikramuddin S, Wolfe B. Clinical Outcomes of Metabolic Surgery: Efficacy of Glycemic Control, Weight Loss, and Remission of Diabetes. Diabetes Care. 2016 Jun;39(6):902-11.

[44] Pories WJ, Caro JF, Flickinger EG, Meelheim HD, Swanson MS. The control of diabetes mellitus (NIDDM) in the morbidly obese with the Greenville Gastric Bypass. Ann Surg 1987;206:316-23.

[45] Buchwald H, Estok R, Fahrbach K, et al. Weight and type 2 diabetes after bariatric surgery: systematic review and meta-analysis. AmJ Med 2009;122:248–256.e5

[46] Sjöström L, Peltonen M, Jacobson P, et al. Association of bariatric surgery with long-term remission of type 2 diabetes and with microvascular and macrovascular complications. JAMA 2014;311:2297–2304

[47] Ding SA, Simonson DC, Wewalka M, et al. Adjustable gastric band surgery or medical management in patients with type 2 diabetes: a randomized clinical trial. J Clin Endocrinol Metab 2015;100:2546–2556

[48] Rubino F, Gagner M, Gentileschi P, Kini S, Fukuyama S, Feng J, Diamond E. The early effect of the Roux-en-Y gastric bypass on hormones involved in body weight regulation and glucose metabolism. Ann Surg. 2004;240:236–242.

[49] le Roux CW, Aylwin SJ, Batterham RL, Borg CM, Coyle F, Prasad V, Shurey S, Ghatei MA, Patel AG, Bloom SR. Gut hormone profiles following bariatric surgery favor an anorectic state, facilitate weight loss, and improve metabolic parameters. Ann Surg. 2006;243:108–114.

[50] Rubino F. Bariatric surgery: effects on glucose homeostasis. Curr Opin Clin Nutr Metab Care 2006;9:497–507.

[51] Vest AR, Heneghan HM, Agarwal S, Schauer PR, Young JB. Bariatric surgery and cardiovascular outcomes: a systematic review. Heart. 2012;98:1763–1777.

[52] Vest AR, Heneghan HM, Schauer PR, Young JB. Surgical management of obesity and the relationship to cardiovascular disease. Circulation 2013;127:945–959

[53] Flum DR, Dellinger EP. Impact of gastric bypass operation on survival: a population-based analysis. J Am Coll Surg. 2004;199:543–551.

[54] Adams TD, Gress RE, Smith SC, Halverson RC, Simper SC, Rosamond WD, Lamonte MJ, Stroup AM, Hunt SC. Long-term mortality after gastric bypass surgery. N Engl J Med. 2007;357:753–761.

[55] Kwok CS, Pradhan A, Khan MA, Anderson SG, Keavney BD, Myint PK, Mamas MA, Loke YK. Bariatric surgery and its impact on cardiovascular disease and mortality: a systematic review and meta-analysis. Int J Cardiol. 2014 Apr 15;173(1):20-8.

[56] MacDonald KG Jr, Long SD, Swanson MS, Brown BM, Morris P, Dohm GL, Pories WJ. The gastric bypass operation reduces the progression and mortality of non-insulin-dependent diabetes mellitus. J Gastrointest Surg. 1997;1:213–220.

[57] Christou NV, Sampalis JS, Liberman M, Look D, Auger S, McLean AP, MacLean LD. Surgery decreases long-term mortality, morbidity, and health care use in morbidly obese patients. Ann Surg. 2004;240:416–423.

[58] Sampalis JS, Sampalis F, Christou N. Impact of bariatric surgery on cardiovascular and musculoskeletal morbidity. Surg Obes Relat Dis. 2006;2:587–591.

[59] Livingston EH, Arterburn D, Schifftner TL, Henderson WG, DePalma RG. National Surgical Quality Improvement Program analysis of bariatric operations: modifiable risk factors contribute to bariatric surgical adverse outcomes. J Am Coll Surg. 2006;203:625–633.

[60] Torquati A, Wright K, Melvin W, Richards W. Effect of gastric bypass operation on Framingham and actual risk of cardiovascular events in class II to III obesity. J Am Coll Surg. 2007;204:776–782.

[61] Sowemimo OA, Yood SM, Courtney J, Moore J, Huang M, Ross R, Mc-Millian U, Ojo P, Reinhold RB. Natural history of morbid obesity without surgical intervention. Surg Obes Relat Dis. 2007;3:73–77.

[62] Peeters A, O'Brien PE, Laurie C, Anderson M, Wolfe R, Flum D, MacInnis RJ, English DR, Dixon J. Substantial intentional weight loss and mortality in the severely obese. Ann Surg. 2007;246:1028–1033.

[63] Maciejewski ML, Livingston EH, Smith VA, Kavee AL, Kahwati LC, Henderson WG, Arterburn DE. Survival among high-risk patients after bariatric surgery. JAMA. 2011;305:2419–2426.

[64] Adams TD, Davidson LE, Litwin SE, Kolotkin RL, LaMonte MJ, Pendleton RC, Strong MB, Vinik R, Wanner NA, Hopkins PN, Gress RE, Walker JM, Cloward TV, Nuttall RT, Hammoud A, Greenwood JL, Crosby RD, McKinlay R,

Simper SC, Smith SC, Hunt SC. Health benefits of gastric bypass surgery after 6 years. JAMA. 2012;308:1122–1131.

[65] Romeo S, Maglio C, Burza MA, Pirazzi C, Sjöholm K, Jacobson P, Svensson PA, Peltonen M, Sjöström L, Carlsson LM. Cardiovascular events after bariatric surgery in obese subjects with type 2 diabetes. Diabetes Care. 2012;35:2613–2617.

[66] Arterburn D, Bogart A, Coleman KJ, Haneuse S, Selby JV, Sherwood NE, et al. Comparative effectiveness of bariatric surgery vs. nonsurgical treatment of type 2 diabetes among severely obese adults. Obes Res Clin Pract. 2013;7(4):e258–68.

[67] Nosso G, Griffo E, Cotugno M, Saldalamacchia G, Lupoli R, Pacini G, Riccardi G, Angrisani L, Capaldo B. Comparative Effects of Roux-en-Y Gastric Bypass and Sleeve Gastrectomy on Glucose Homeostasis and Incretin Hormones in Obese Type 2 Diabetic Patients: A One-Year Prospective Study. Horm Metab Res. 2016 May;48(5):312-7.

[68] Mari A, Pacini G, Murphy E, Ludvik B, Nolan JJ. A model-based method for assessing insulin sensitivity from the oral glucose tolerance test. Diabetes Care 2001; 24: 539–548

[69] Van Cauter E, Mestrez F, Sturis J, Polonsky KS. Estimation of insulin secretion rates from C-peptide levels: comparison of individual and standard kinetic parameters for C-peptide clearance. Diabetes 1992; 41: 368–377

[70] Ahrén B, Pacini G. Impaired adaptation of first-phase insulin secretion in postmenopausal women with glucose intolerance. Am J Physiol 1997; 273 (4 Pt 1): E701–E707

[71] Madsbad S, Holst JJ. GLP-1 as a mediator in the remission of type 2 diabetes after gastric bypass and sleeve gastrectomy surgery. Diabetes 2014; 63: 3172–3174

[72] Camastra S,GastaldelliA,Mari A, et al. Early and longer termeffects of gastric bypass surgery on tissue-specific insulin sensitivity and beta cell function in morbidly obese patients with and without type 2 diabetes. Diabetologia. 2011;54:2093–102.

[73] Baggio LL, Drucker DJ. Biology of incretins: GLP-1 and GIP. Gastroenterology. 2007;132(6):2131–57.

[74] Madsbad S. The role of GLP-1 impairment in obesity and potential therapeutic implications. Diabetes Obes Metab. 2013.

[75] Kannel WB, Vasan RS. Triglycerides as vascular risk factors: new epidemiologic insights. Curr Opin Cardiol. 2009;24(4):345–50.

[76] Griffo E, Nosso G, Lupoli R, Cotugno M, Saldalamacchia G, Vitolo G, Angrisani L, Cutolo PP, Rivellese AA, Capaldo B. Early improvement of

postprandial lipemia after bariatric surgery in obese type 2 diabetic patients. Obes Surg. 2014 May;24(5):765-70.

[77] Griffo E, Cotugno M, Nosso G, Saldalamacchia G, Mangione A, Angrisani L, Rivellese AA, Capaldo B. Effects of Sleeve Gastrectomy and Gastric Bypass on Postprandial Lipid Profile in Obese Type 2 Diabetic Patients: a 2-Year Follow-up. Obes Surg. 2016 Jun;26(6):1247-53.

[78] Benetti A, Del Puppo M, Crosignani A, et al. Cholesterol metabolism after bariatric surgery in grade 3 obesity: differences between malabsorptive and restrictive procedures. Diabetes Care. 2013;36(6):1443–7.

[79] Saboya C, Arasaki CH, Matos D, et al. Relationship between the preoperative body mass index and the resolution of metabolic syndrome following Roux-en-Y gastric bypass. Metab Syndr Relat Disord. 2012;10(4):292–6.

[80] Tinahones FJ, Queipo-Ortuño MI, Clemente-Postigo M, et al. Postprandial hypertriglyceridemia predicts improvement in insulin resistance in obese patients after bariatric surgery. Surg Obes Relat Dis. 2013;9(2):213–8.

[81] Moustarah F, Gilbert A, Després JP, Tchernof A. Impact of gastrointestinal surgery on cardiometabolic risk. Curr Atheroscler Rep. 2012;14(6):588–96.

[82] Ben-Shlomo S, Zvibel I, Shnell M, et al. Glucagon-like peptide-1 reduces hepatic lipogenesis via activation ofAMP-activated protein kinase. J Hepatol. 2011;54(6):1214–23.

[83] Svegliati-Baroni G, Saccomanno S, Rychlicki C, et al. Glucagonlike peptide-1 receptor activation stimulates hepatic lipid oxidation and restores hepatic signalling alteration induced by a high-fat diet in nonalcoholic steatohepatitis. Liver Int. 2011;31(9):1285–97.

[84] Batterham RL, Cummings DE. Mechanisms of Diabetes Improvement Following Bariatric/Metabolic Surgery. Diabetes Care 2016 39 893-901

[85] Jones KL, Horowitz M, Carney BI, Wishart JM, Guha S, Green L. Gastric emptying in early noninsulin-dependent diabetes mellitus. Journal of nuclear medicine: official publication, Society of Nuclear Medicine 1996 37 1643-1648

[86] Abrahamsson N, Edén Engström B, Sundbom M, Karlsson FA. Hypoglycemia in everyday life after gastric bypass and duodenal switch. Eur J Endocrinol 2015 173 91-100.

[87] Patti ME, Goldfine AB. The rollercoaster of post-bariatric hypoglycaemia. The Lancet Diabetes & Endocrinology 2016 4 94-96

[88] Ritz P, Vaurs C, Barigou M, Hanaire H. Hypoglycaemia after gastric bypass: mechanisms and treatment. Diabetes, Obesity and Metabolism 2016 18 217-23

[89] Service FJ. Glucose variability. Diabetes 2013 62 1398-1404

[90] Hirsch IB. Glycemic Variability and Diabetes Complications: Does It Matter? Of Course It Does! Diabetes Care 2015 38 1610-1614

[91] Bergenstal RM. Glycemic Variability and Diabetes Complications: Does It Matter? Simply Put, There Are Better Glycemic Markers! Diabetes Care 2015 38 1615-1621

[92] Gorst C, Kwok CS, Aslam S, Buchan I, Kontopantelis E, Myint PK, Heatlie G, Loke Y, Rutter MK, Mamas MA. Long-term Glycemic Variability and Risk of Adverse Outcomes: A Systematic Review and Meta-analysis. Diabetes Care 2015 38 2354-2369

[93] Monnier L, Mas E, Ginet C, Michel F, Villon L, Cristol JP, Colette C. Activation of oxidative stress by acute glucose fluctuations compared with sustained chronic hyperglycemia in patients with type 2 diabetes. Jama 2006 295 1681-1687

[94] Kovatchev B, Cobelli C. Glucose Variability: Timing, Risk Analysis, and Relationship to Hypogly-cemia in Diabetes. Diabetes Care 2016 39 502-510

[95] Nalysnyk L, Hernandez-Medina M, Krishnarajah G. Glycaemic variability and complications in pa-tients with diabetes mellitus: evidence from a systematic review of the literature. Diabetes, Obesity and Metabolism. 2010 12 288-98

[96] Chang CM, Hsieh CJ, Huang JC, Huang IC. Acute and chronic fluctuations in blood glucose levels can increase oxidative stress in type 2 diabetes mellitus. Acta Diabetologica 2012 49 Suppl 1 S171-177

[97] Zenari L, Marangoni A. What are the preferred strategies for control of glycaemic variability in pa-tients with type 2 diabetes mellitus? Diabetes, Obesity and Metabolism 2013 15 Suppl 2 17-25

[98] He YL, Foteinos G, Neelakantham S, Mattapalli D, Kulmatycki K, Forst T, Taylor A. Differential effects of vildagliptin and glimepiride on glucose fluctuations in patients with type 2 diabetes melli-tus assessed using continuous glucose monitoring. Diabetes, Obesity and Metabolism 2013 15 1111-9

[99] Halperin F, Patti ME, Skow M, Bajwa M, Goldfine AB. Continuous glucose monitoring for evalua-tion of glycemic excursions after gastric bypass. Journal of obesity 2011 2011 869-536

[100] Jimenez A, Ceriello A, Casamitjana R, Flores L, Viaplana-Masclans J, Vidal J. Remission of type 2 diabetes after Roux-en-Y gastric bypass or sleeve gastrectomy is associated with a distinct glycemic profile. Annals of Surgery 2015 261 316-322

[101] Yu H, Zhou J, Bao Y, Pin Zhang, Lu W, Jia W. "Dual-remission" after Roux-en-Y gastric bypass surgery: Glycemic variability cannot always be improved in

Chinese obese patients with type 2 diabetes. *Surg Obes Relat Dis* 2016 12 1312-1319

[102] Buscemi S, Cosentino L, Rosafio G, Morgana M, Mattina A, Sprini D, Verga S, Rini GB. Effects of hypocaloric diets with different glycemic indexes on endothelial function and glycemic variability in overweight and in obese adult patients at increased cardiovascular risk. Clinical Nutrition 2013 32 346-352

[103] Augustin LS, Kendall CW, Jenkins DJ, Willett WC, Astrup A, Barclay AW, Björck I, Brand-Miller JC, Brighenti F, Buyken AE, Ceriello A, La Vecchia C, Livesey G, Liu S, Riccardi G, Rizkalla SW, Sievenpiper JL, Trichopoulou A, Wolever TM, Baer-Sinnott S, Poli A. Glycemic index, glycemic load and glycemic response: An International Scientific Consensus Summit from the International Carbohydrate Quality Consortium (ICQC). Nutrition, Metabolism, and Cardiovascular Diseases: NMCD 2015 25 795-815

[104] Busso N, Hamilton JA. Extravascular coagulation and the plasminogen activator/plasmin system in rheumatoid arthritis. Arthritis Rheum 2002; 46: 2268-79.

[105] Samad F, Ruf W. Inflammation, obesity, and thrombosis. Blood 2013; 122: 3415-22.

[106] Poirier P, Giles TD, Bray GA, et al. Obesity and cardiovascular disease: pathophysiology, evaluation, and effect of weight loss. Arterioscler Thromb Vasc Biol 2006; 26: 968-76.

[107] Lupoli R, Milone M, Di Minno A, Maietta P, Ambrosino P, Musella M, Di Minno MN.Haemostatic and fibrinolytic changes in obese subjects undergoing bariatric surgery: the effect of different surgical procedures. Blood Transfus. 2015 Jul;13(3):442-7.

[108] Fried M, Yumuk V, Oppert JM, Scopinaro N, Torres A, Weiner R, Yashkov Y, Frühbeck G; International Federation for the Surgery of Obesity and Metabolic Disorders—European Chapter (IFSO-EC) and European Association for the Study of Obesity (EASO). Interdisciplinary European guidelines on metabolic and bariatric surgery. Obes Surg. 2014 Jan;24(1):42-55.

[109] Basu TK, Donaldson D. Intestinal absorption in health and disease: micronutrients. Best Pract Res Clin Gastroenterol. 2003 Dec;17(6):957-79.

[110] Thorell A. Clinical Nutrition University: Nutritional support after bariatric surgery e-SPEN, the European e-Journal of Clinical Nutrition and Metabolism. 2011;6(2):96–100.

[111] Slater GH, Ren CJ, Siegel N, Williams T, Barr D, Wolfe B, Dolan K, Fielding GA. Serum fat-soluble vitamin deficiency and abnormal calcium metabolism after malabsorptive bariatric surgery. J Gastrointest Surg. 2004 Jan;8(1):48-55.

[112] Ziegler O, Sirveaux MA, Brunaud L, Reibel N, Quilliot D. Medical follow up after bariatric surgery: nutritional and drug issues. General recommendations for the prevention and treatment of nutritional deficiencies. Diabetes Metab. 2009 Dec;35(6 Pt 2):544-57.

[113] Gringeri A, Mannucci PM; Italian Association of Haemophilia Centres. Italian guidelines for the diagnosis and treatment of patients with haemophilia and inhibitors. Haemophilia. 2005 Nov;11(6):611-9.

[114] Di Minno MN, Dolce A, Mariani G; STER Study Group. Bleeding symptoms at disease presentation and prediction of ensuing bleeding in inherited FVII deficiency. Thromb Haemost. 2013 Jun;109(6):1051-9.

[115] Sanson BJ, Simioni P, Tormene D, et al. The incidence of venous thromboembolism in asymptomatic carriers of a deficiency of antithrombin, protein C, or protein S: a prospective cohort study. Blood 1999; 94: 3702–3706.

[116] Bucciarelli P, Passamonti SM, Biguzzi E, Gianniello F, Franchi F, Mannucci PM, Martinelli I. Low borderline plasma levels of antithrombin, protein C and protein S are risk factors for venous thromboembolism. J Thromb Haemost. 2012 Sep;10(9):1783-91.

[117] Di Minno MN, Dentali F, Lupoli R, Ageno W. Mild Antithrombin Deficiency and the Risk of Recurrent Venous Thromboembolism: A Prospective Cohort Study. Circulation. 2013 Oct 21. [Epub ahead of print]

[118] Poulose BK, Griffin MR, Zhu Y, Smalley W, Richards WO, Wright JK et al. National analysis of adverse patient safety for events in bariatric surgery. Am Surg 2005; 71: 406–413.

[119] Caballero AE. Endothelial dysfunction in obesity and insulin resistance: a road to diabetes and heart disease. Obes Res. 2003;11:1278-1289.

[120] Williams IL, Chowienczyk PJ, Wheatcroft SB, Patel AG, Sherwood RA, Momin A, et al. Endothelial function and weight loss in obese humans. Obes Surg. 2005;15:1055-1060.

[121] Lorenz MW, Markus HS, Bots ML, Rosvall M, Sitzer M. Prediction of clinical cardiovascular events with carotid intima-media thickness: a systematic review and meta-analysis. Circulation. 2007;115:459–467.

[122] Chan SY, Mancini GB, Kuramoto L, Schulzer M, Frohlich J, Ignaszewski A. The prognostic importance of endothelial dysfunction and carotid atheroma burden in patients with coronary artery disease. J Am Coll Cardiol. 2003;42:1037–1043.

[123] Bots ML, Grobbee DE. Intima media thickness as a surrogate marker for generalised atherosclerosis. Cardiovasc Drugs Ther. 2002;16: 341-351.

[124] de Groot E, Hovingh GK, Wiegman A, Duriez P, Smit AJ, Fruchart JC, et al. Measurement of arterial wall thickness as a surrogate marker for atherosclerosis. Circulation. 2004; 109(23 Suppl 1):III33-III38.

[125] O'Leary DH, Polak JF, Kronmal RA, Manolio TA, Burke GL, Wolfson SK Jr. Carotid-artery intima and media thickness as a risk factor for myocardial infarction and stroke in older adults. Cardiovascular Health Study Collaborative Research Group. N Engl J Med. 1999; 340:14-22.

[126] Chambless LE, Heiss G, Folsom AR, Rosamond W, Szklo M, Sharrett AR, et al. Association of coronary heart disease incidence with carotid arterial wall thickness and major risk factors: the Atherosclerosis Risk in Communities (ARIC) Study, 1987-1993. Am J Epidemiol. 1997;146:483-494.

[127] Yeboah J, Folsom AR, Burke GL, Johnson C, Polak JF, Post W, et al. Predictive value of brachial flow-mediated dilation for incident cardiovascular events in a population-based study: the multi-ethnic study of atherosclerosis. Circulation. 2009;120:502-509.

[128] Akamatsu D, Sato A, Goto H, Watanabe T, Hashimoto M, Shimizu T, et al. Nitroglycerin-mediated vasodilatation of the brachial artery may predict long-term cardiovascular events irrespective of the presence of atherosclerotic disease. J Atheroscler Thromb. 2010;17:1266-1274.

[129] Lupoli R, Di Minno MN, Guidone C, Cefalo C, Capaldo B, Riccardi G, Mingrone G. Effects of bariatric surgery on markers of subclinical atherosclerosis and endothelial function: a meta-analysis of literature studies. Int J Obes (Lond). 2016 Mar;40(3):395-402.

[130] Gokce N, Vita JA, McDonnell M, Forse AR, Istfan N, Stoeckl M, et al. Effect of medical and surgical weight loss on endothelial vasomotor function in obese patients. Am J Cardiol. 2005; 95: 266–268.

[131] Lind L, Zethelius B, Sundbom M, Edén Engström B, Karlsson FA. Vasoreactivity is rapidly improved in obese subjects after gastric bypass surgery. Int J Obes (Lond). 2009;33:1390-1395.

[132] Sarmento PL, Plavnik FL, Zanella MT, Pinto PE, Miranda RB, Ajzen SA. Association of carotid intima-media thickness and cardiovascular risk factors in women pre- and post-bariatric surgery. Obes Surg. 2009; 19:339-344.

[133] Habib P, Scrocco JD, Terek M, Vanek V, Mikolich JR. Effects of bariatric surgery on inflammatory, functional and structural markers of coronary atherosclerosis. Am J Cardiol. 2009;104:1251-1255.

[134] Brethauer SA, Heneghan HM, Eldar S, Gatmaitan P, Huang H, Kashyap S, et al. Early effects of gastric bypass on endothelial function, inflammation, and cardiovascular risk in obese patients. Surg Endosc. 2011; 25:2650-2659.

[135] Saleh MH, Bertolami MC, Assef JE, Taha MI, de Freitas W Jr, Petisco AC, et al. Improvement of atherosclerotic markers in non-diabetic patients after bariatricsurgery. Obes Surg. 2012; 22:1701-1707.

[136] Tschoner A, Sturm W, Gelsinger C, Ress C, Laimer M, Engl J, et al. Long-term effects of weight loss after bariatric surgery on functional and structural markers of atherosclerosis. Obesity (Silver Spring). 2013; 21:1960-1965.

[137] Sturm W, Tschoner A, Engl J, Kaser S, Laimer M, Ciardi C, et al. Effect of bariatric surgery on both functional and structural measures of premature atherosclerosis. Eur Heart J. 2009;30:2038-2043.

[138] Nerla R, Tarzia P, Sestito A, Di Monaco A, Infusino F, Matera D, et al. Effect of bariatric surgery on peripheral flow-mediated dilation and coronary microvascular function. Nutr Metab Cardiovasc Dis. 2012; 22:626-634.

[139] García G, Bunout D, Mella J, Quiroga E, de la Maza MP, Cavada G, et al. Bariatric surgery decreases carotid intima-media thickness in obese subjects. Nutr Hosp. 2013;28:1102-1108.

[140] Brethauer SA, Heneghan HM, Eldar S, Gatmaitan P, Huang H, Kashyap S, et al. Early effects of gastric bypass on endothelial function, inflammation, and cardiovascular risk in obese patients. Surg Endosc. 2011; 25:2650-2659.

[141] Gloy VL, Briel M, Bhatt DL, Kashyap SR, Schauer PR, Mingrone G, et al. Bariatric surgery versus non-surgical treatment for obesity: a systematic review and meta-analysis of randomised controlled trials. BMJ. 2013; 347: f5934

[142] John S, Hoegerl C. Nutritional deficiencies after gastric bypass surgery. J Am Osteopath Assoc. 2009 Nov;109(11):601-4.

[143] Xanthakos SA. Nutritional deficiencies in obesity and after bariatric surgery. Pediatr Clin North Am. 2009 Oct;56(5):1105-21.

[144] Zalesin KC, Miller WM, Franklin B, Mudugal D, Rao Buragadda A, Boura J, Nori-Janosz K, Chengelis DL, Krause KR, McCullough PA. Vitamin a deficiency after gastric bypass surgery: an underreported postoperative complication. J Obes. 2011;2011. pii: 760695.

[145] Liu C, Wu D, Zhang JF, Xu D, Xu WF, Chen Y, Liu BY, Li P, Li L. Changes in Bone Metabolism in Morbidly Obese Patients After Bariatric Surgery: A Meta-Analysis. Obes Surg. 2016 Jan;26(1):91-7.

[146] Weng TC, Chang CH, Dong YH, Chang YC, Chuang LM. Anaemia and related nutrient deficiencies after Roux-en-Y gastric bypass surgery: a systematic review and meta-analysis. BMJ Open. 2015 Jul 16;5(7):e006964.

[147] Ben-Porat T, Elazary R, Yuval JB, Wieder A, Khalaileh A, Weiss R. Nutritional deficiencies after sleeve gastrectomy: can they be predicted preoperatively?Surg Obes Relat Dis. 2015 Sep-Oct;11(5):1029-36.

[148] Blume CA, Boni CC, Casagrande DS, Rizzolli J, Padoin AV, Mottin CC. Nutritional profile of patients before and after Roux-en-Y gastric bypass: 3-year follow-up. Obes Surg. 2012 Nov;22(11):1676-85. doi: 10.1007/s11695-012-0696-y.

[149] Carrodeguas L, Kaidar-Person O, Szomstein S, Antozzi P, Rosenthal R. Preoperative thiamine deficiency in obese population undergoing laparoscopicbariatric surgery. Surg Obes Relat Dis. 2005 Nov-Dec;1(6):517-22; discussion 522.

[150] Censani M, Stein EM, Shane E, Oberfield SE, McMahon DJ, Lerner S, Fennoy I. Vitamin D Deficiency Is Prevalent in Morbidly Obese Adolescents Prior to Bariatric Surgery. ISRN Obes. 2013;2013.

[151] Dagan SS, Zelber-Sagi S, Webb M, Keidar A, Raziel A, Sakran N, Goitein D, Shibolet O. Nutritional Status Prior to Laparoscopic Sleeve Gastrectomy Surgery. Obes Surg. 2016 Sep;26(9):2119-26. doi: 10.1007/s11695-016-2064-9. PubMed PMID: 26797718.

[152] Damms-Machado A, Friedrich A, Kramer KM, Stingel K, Meile T, Küper MA, Königsrainer A, Bischoff SC. Pre- and postoperative nutritional deficiencies in obese patients undergoing laparoscopic sleeve gastrectomy. Obes Surg. 2012 Jun;22(6):881-9.

[153] de Luis DA, Pacheco D, Izaola O, Terroba MC, Cuellar L, Cabezas G. Micronutrient status in morbidly obese women before bariatric surgery. Surg Obes Relat Dis. 2013 Mar-Apr;9(2):323-7

[154] Ernst B, Thurnheer M, Schmid SM, Schultes B. Evidence for the necessity to systematically assess micronutrient status prior to bariatric surgery. Obes Surg. 2009 Jan;19(1):66-73.

[155] Ewang-Emukowhate M, Harrington DJ, Botha A, McGowan B, Wierzbicki AS. Vitamin K and other markers of micronutrient status in morbidly obese patients before bariatric surgery. Int J Clin Pract. 2015 Jun;69(6):638-42. doi: 10.1111/ijcp.12594. Epub 2014 Dec 11.

[156] Flancbaum L, Belsley S, Drake V, Colarusso T, Tayler E. Preoperative nutritional status of patients undergoing Roux-en-Y gastric bypass for morbid obesity. J Gastrointest Surg. 2006 Jul-Aug;10(7):1033-7.

[157] Gehrer S, Kern B, Peters T, Christoffel-Courtin C, Peterli R. Fewer nutrient deficiencies after laparoscopic sleeve gastrectomy (LSG) than after laparoscopic Roux-Y-gastric bypass (LRYGB)-a prospective study. Obes Surg. 2010 Apr;20(4):447-53. doi: 10.1007/s11695-009-0068-4. Epub 2010 Jan 26.

[158] Gemmel K, Santry HP, Prachand VN, Alverdy JC. Vitamin D deficiency in preoperative bariatric surgery patients. Surg Obes Relat Dis. 2009 Jan-Feb;5(1):54-9.

[159] Gerig R, Ernst B, Wilms B, Thurnheer M, Schultes B. Preoperative nutritional deficiencies in severely obese bariatric candidates are not linked to gastric Helicobacter pylori infection. Obes Surg. 2013 May;23(5):698-702. doi: 10.1007/s11695-013-0878-2.

[160] Gillon S, Jeanes YM, Andersen JR, Våge V. Micronutrient Status in Morbidly Obese Patients Prior to Laparoscopic Sleeve Gastrectomy and Micronutrient Changes 5 years Post-surgery. Obes Surg. 2016 Aug 5.

[161] Gobato RC, Seixas Chaves DF, Chaim EA. Micronutrient and physiologic parameters before and 6 months after RYGB. Surg Obes Relat Dis. 2014 Sep-Oct;10(5):944-51.

[162] Jin J, Stellato TA, Hallowell PT, Schuster M, Graf K, Wilhelm S. Utilization of preoperative patient factors to predict postoperative vitamin D deficiency for patients undergoing gastric bypass. J Gastrointest Surg. 2009 Jun;13(6):1052-7.

[163] Lefebvre P, Letois F, Sultan A, Nocca D, Mura T, Galtier F. Nutrient deficiencies in patients with obesity considering bariatric surgery: a cross-sectional study. Surg Obes Relat Dis. 2014 May-Jun;10(3):540-6.

[164] Madan AK, Orth WS, Tichansky DS, Ternovits CA. Vitamin and trace mineral levels after laparoscopic gastric bypass. Obes Surg. 2006 May;16(5):603-6.

[165] Moizé V, Deulofeu R, Torres F, de Osaba JM, Vidal J. Nutritional intake and prevalence of nutritional deficiencies prior to surgery in a Spanish morbidly obese population. Obes Surg. 2011 Sep;21(9):1382-8. doi: 10.1007/s11695-011-0360-y.

[166] Papamargaritis D, Aasheim ET, Sampson B, le Roux CW.Copper, selenium and zinc levels after bariatric surgery in patients recommended to take multivitamin-mineral supplementation. J Trace Elem Med Biol. 2015;31:167-72. doi: 10.1016/j.jtemb.2014.09.005. Epub 2014 Sep 16.

[167] Peterson LA, Cheskin LJ, Furtado M, Papas K, Schweitzer MA, Magnuson TH, Steele KE. Malnutrition in Bariatric Surgery Candidates: Multiple Micronutrient Deficiencies Prior to Surgery. Obes Surg. 2016 Apr;26(4):833-8. doi: 10.1007/s11695-015-1844-y. PubMed PMID: 26297429.

[168] Sánchez A, Rojas P, Basfi-Fer K, Carrasco F, Inostroza J, Codoceo J, Valencia A, Papapietro K, Csendes A, Ruz M. Micronutrient Deficiencies in Morbidly Obese Women Prior to Bariatric Surgery. Obes Surg. 2016 Feb;26(2):361-8.

[169] Schiavo L, Scalera G, Pilone V, De Sena G, Capuozzo V, Barbarisi A. Micronutrient Deficiencies in Patients Candidate for Bariatric Surgery: A Prospective, Preoperative Trial of Screening, Diagnosis, and Treatment. Int J Vitam Nutr Res. 2016 May 10:1-8.

[170] Schweiger C, Weiss R, Berry E, Keidar A. Nutritional deficiencies in bariatric surgery candidates. Obes Surg. 2010 Feb;20(2):193-7.

[171] Skroubis G, Sakellaropoulos G, Pouggouras K, Mead N, Nikiforidis G, Kalfarentzos F. Comparison of nutritional deficiencies after Roux-en-Y gastric bypass and after biliopancreatic diversion with Roux-en-Y gastric bypass. Obes Surg. 2002 Aug;12(4):551-8

[172] Toh SY, Zarshenas N, Jorgensen J. Prevalence of nutrient deficiencies in bariatric patients. Nutrition. 2009 Nov-Dec;25(11-12):1150-6

[173] van Rutte PW, Aarts EO, Smulders JF, Nienhuijs SW. Nutrient deficiencies before and after sleeve gastrectomy. Obes Surg. 2014 Oct;24(10):1639-46.

[174] Wang C, Guan B, Yang W, Yang J, Cao G, Lee S. Prevalence of electrolyte and nutritional deficiencies in Chinese bariatric surgery candidates. Surg Obes Relat Dis. 2016 Mar-Apr;12(3):629-34.

[175] Wolf E, Utech M, Stehle P, Büsing M, Stoffel-Wagner B, Ellinger S. Preoperative micronutrient status in morbidly obese patients before undergoing bariatric surgery: results of a cross-sectional study. Surg Obes Relat Dis. 2015 Sep-Oct;11(5):1157-63.

[176] Stein J, Stier C, Raab H, Weiner R. Review article: The nutritional and pharmacological consequences of obesity surgery. Aliment Pharmacol Ther. 2014 Sep;40(6):582-609.

[177] Bjelakovic G, Gluud Lise L, Nikolova D, et al. Vitamin D supplementation for prevention of cancer in adults. Cochrane Database Syst Rev. 2008;4:1–16.

[178] Szodoray P, Zeher M, Bodolay E, et al. The complex role of vitamin D in autoimmune diseases. Scand J Immunol. 2008;68(3):261–70.

[179] Mitri J, Pittas AG. Vitamin D and diabetes. Endocrinol Metab Clin N Am. 2014;43(1):205–32

[180] Ybarra J, Sanchez-Hernandez J, Perez A. Hypovitaminosis D and morbid obesity. Nurs Clin North Am. 2007;42(1):19–27.

[181] Lanzarini E, Nogués X, Goday A, et al. High-dose vitamin D supplementation is necessary after bariatric surgery: a prospective 2- year follow-up study. Obes Surg. 2015;1-6

[182] Drincic AT, Armas LAG, Van Diest EE, et al. Volumetric dilution, rather than sequestration best explains the low vitamin D status of obesity. Obesity. 2012;20(7):1444–8.

[183] Wortsman J, Matsuoka LY, Chen TC, et al. Decreased bioavailability of vitamin D in obesity. Am J Clin Nutr. 2000;72(3):690–3.

[184] Jiang H, Xiong DH, Guo YF, et al. Association analysis of vitamin D-binding protein gene polymorphisms with variations of obesityrelated traits in Caucasian nuclear families. Int J Obes. 2007;31(8): 1319–24.

[185] Ochs-Balcom HM, Chennamaneni R, Millen AE, et al. Vitamin D receptor gene polymorphisms are associated with adiposity phenotypes. Am J Clin Nutr. 2011;93(1):5–10.