DOTTORATO DI RICERCA IN SCIENZE BIOMEDICHE

CICLO XXXIV

COORDINATORE Prof. Fabrizio Chiti

Do exist specific predictors of outcome in terms of long-term weight-loss after bariatric surgery?
Visceral adipose tissue adiponectin and serum triglycerides can predict excess weight loss after bariatric surgery in female subjects with severe obesity.

Settore Scientifico Disciplinare MED/50

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INDEX

ABSTRACT

Objective: Bariatric surgery does not always result in satisfactory excess weight loss (EWL) in severe obesity. Given the economic and clinical costs of bariatric surgery failure, defining predictors of successful EWL represents a relevant clinical issue for the health system to select patients benefiting from operation.

Methods: From September 2017 to 2021 130 severe obesity patients undergoing laparoscopic bariatric surgery were enrolled. Pre-surgery evaluations included anthropometrics and biochemical analyses.. Follow-ups occurred at 6, 12, 18, and 24 months, tracking weight, BMI, and comorbidities. Statistical correlation was performed in order to find a predictor of EWL after Surgery. Furthermore, we built a pilot study involving a subgroup of43 patients investigating adiponectin levels in subcutaneous (SAT) and visceral adipose tissue (VAT) samples that were obtained during bariatric surgery. We analyze possible clinical parameter predictors assessed at baseline of EWL at different times of follow up. In a subgroup of 43 patients, by ELISA and Western blot analyses, we assessed the predicting value of pre-operative adiponectin (APN) locally produced in abdominal visceral (VAT) and subcutaneous (SAT) adipose tissue versus plasma levels as a novel sex-linked biomarker of EWL at different time points of follow up (6-24 months) after bariatric surgery.

Results: In the entire cohort, we found at multivariate analysis HbA1 and NASH as well as triglycerides as good independent predictors of EWL at short and long time intervals, respectively, and only in female subjects. In the subgroup of operated patients where tissue adiponectin was available, we found that VAT-APN was lower in females and represented the only marker significantly correlated with EWL. In females, VAT-APN in the distribution upper quartile but not baseline BMI retained a statistically significant correlation with EWL at any time points (6-24 months) at multivariate analysis. The best VAT-APN cut-off value to predict 95% EWL at 12 months from surgery (98% accuracy, 100% sensitivity, 94% specificity, p=0.010) was 5.1µg/mg.

Conclusions: In conclusion, our findings indicate that in females affected by severe obesity, novel predictors of EWL can be identified. In particular, APN produced in the abdominal VAT rather than its circulating or subcutaneous levels can predict EWL after bariatric surgery as an independent factor only in the female sex. Similarly, basal TG and HBA1c, as well as NASH revealed to be putative factors that can predict EWL in females subjects thus contributing to better identify those patients who could much benefit from surgery.

Chapter 1
INTRODUCTION

The prevalence of severe obesity and its associated life-threatening comorbidities has exponentially increased worldwide in the last decades, also worriedly interesting paediatric ages. (*Pinhas-Hamiel et al. 2022*)

The health consequences of obesity are well documented and raised Body Mass Index (BMI) is an established risk factor for increased mortality and morbidity rates. (*Whitlock et al. 2009*)

Elevated BMI and, in particular, Visceral Adipose Tissue (VAT) increase, is often associated with cardiovascular and metabolic risk factors such as hypertension, hyperglycemia, and atherogenic dyslipidemia. This effect appears to be due VAT incrementation. In fact VAT is metabolically active releasing pro-inflammatory cytokines and fatty acids in the portal system with harmful effects both systemic and hepatic. (*Rytka et al. 2011*)

The association of these factors, hypertension, hyperglycemia, and atherogenic dyslipidemia, defines the concept of Metabolic Syndrome (MetS) and identifies a diagnostic category able to predict cardiovascular disease and type 2 diabetes mellitus (T2DM). (*Ford 2005*)

MetS has been recognized to be a complication of adipose tissue dysfunction. Adipose tissue dysfunction starts when in the face of a nutritional overload there is a failure to accommodate all nutrients. (*Baglioni et al. 2009*) Nonalcoholic fatty liver disease (NAFLD) is often considered a hepatic manifestation of MetS and also results associated with increased risk of diabetes and cardiovascular disease. (*Rector et al. 2008*) Indeed, it has also been proposed that NAFLD should be included in the definition of MetS. (*Musso et al. 2008*)

Bariatric surgery should be considered as the elective strategy for patients with body mass index (BMI)≥40 kg/m^2 or BMI≥35–40 kg/m^2 associated with comorbidities, as it results in a rapid and important weight loss associated with a significant improvement of the comorbidities as type 2 diabetes and MetS (*Di Lorenzo et al. 2020*) and a decrease in obesity-related long-term mortality. (*Adams et al. 2018*)

A large Scandinavian population-based cohort study reported, however, that patients with severe obesity obtained a long-lasting reduction in the use of lipid-lowering and antidiabetic medications but only a transient effect for cardiovascular medications after bariatric surgery. (*Kauppila et al. 2023*) Indeed, the response to surgery is not always optimal resulting in insufficient weight loss (IWL= % EWL less than 50%) or weight regain (WR= EWL percentage increases >25% from the minimum weight or gain >15% of total weight lost initially) even at early follow up. (*Yarigholi et al. 2022*) Recent reviews evaluating the current knowledge about mechanisms and predictors of WR and IWL after bariatric surgery, came to the conclusion that these are still poorly characterized and need further investigation. (*El Ansari et al. 2021; Pokala et al. 2023*) Given the economic and clinical costs of failure of bariatric surgery in these patients, searching for predictors of successful and durable weight loss represents a relevant clinical issue for the health system. (*Athanasiadis et al. 2021*)

Circulating levels of hormones involved in the control of energy homeostasis and metabolism, such as the gastric product, ghrelin, and the adipose tissue (AT) adipokines, leptin and adiponectin (APN), have been demonstrated to be associated with weight variations. (*Faraj et al. 2003*) Both plasmatic ghrelin and APN have been suggestive as predictive of the weight loss and of the metabolic amelioration observed post-surgery. (*Williams et al. 2016; Faraj et al. 2003*) However, circulating levels of APN do not reflect the differences in the hormone production and functional significance at the level of different fat depots, being the abdominal visceral AT the most susceptible to the dysregulation occurring in obesity and the target of the main remodelling during weight loss.

Local APN production in AT may represent a more penetrating and predictive marker of the effect of bariatric surgery, also reflecting potential differences between the two sexes.

Therefore, in the present paper, we assessed the putative value of pre-operative APN levels locally produced in visceral (VAT) and subcutaneous (SAT) AT, and compared it with APN plasma levels as sex-linked predictors of excess weight loss (EWL) reached after bariatric surgery.

1.1 Obesity: Classification and Diagnosis

The WHO defines overweight and obesity as conditions in which there is an accumulation of excessive adipose tissue that causes damage to health. (*Organization WHO. Obesity and Overweight fact sheet. 2018)*

An approximate and worldwide used index for quantifying obesity is the Body Mass Index (BMI). This is defined as an individual's weight in kilograms divided by the square of their height in meters: BMI= weight (kg)/height^2 (m^2) *Figure 1.1* shows the Obesity classification according to BMI

Classes	BMI
Under weight	<18,49
Normal weight	18,5 – 24,99
Overweight	25-29,99
Obesity type I	30-34,99
Obesity type II	35-39,99
Obesity type III	>40

Figure 1,1: Obesity classification according to BMI

This index, while useful, has some limitations. First of all, it doesn't take body composition into account. Knowing this allows us to discriminate between an excess of adipose tissue and excess weight due to muscle hypertrophy or water retention. Secondly, BMI does not take into account the distribution of adipose tissue within the body. (*Kang et al. 2023*)

In fact, we know that there are substantial differences in the function of the various fat deposits. More than 80% of total body fat is stored in the abdominal and gluteal-femoral subcutaneous region, which acts as a protective barrier against external mechanical stress, dermal inflammatory processes and heat dispersion. The remaining 20% is stored intraperitoneally (omental, mesenteric and epiploic deposits) and retroperitoneally and forms visceral adipose tissue which, as we have already said, has a metabolic function and is responsible for an increase in cardiovascular risk leading to insulin resistance, dyslipidemia, hypertension, increased inflammatory markers and thrombotic diathesis. (*Alberti et al. 2009; Chianelli et al. 2023*)

Therefore, knowing body composition and fat distribution is useful for assessing the risk of the individual. To overcome these limitations, methods such as bioimpedance measurement, skin fold measurement, waist circumference and waist-to-hip ratio (WHR) can be combined with BMI to estimate body composition, degree and distribution of obesity:).

The bioelectrical impedance analyzer (BIA), also called bioimpedance analysis, is based on the principle that the various body components have a greater or lesser water content and therefore present a different resistance to the passage of an electric current which is analyzed by the device. (*Long et al. 2019*)

Skinfold measurement is carried out using an instrument called a skinfold caliper which allows the subcutaneous fat to be assessed by detecting the thickness of standardized skin folds (bicipital, triceps, subscapular and suprailiac). Waist circumference, measured at the midpoint between the lower edge of the last palpable ribs and the upper part of the iliac crest, is a useful variable for assessing the amount of abdominal fat. (*Lean et al 1995****;*** *Long et al. 2019*)

A waist circumference of 102 cm in men and 88 cm in European women determines an increase in cardiovascular risk and falls within the diagnostic criteria of metabolic syndrome together with hypertriglyceridemia (>150 mg/dl) or ongoing pharmacological treatment, low HDL cholesterol (men <40 mg/dl, women <50 mg/dl) or specific pharmacological therapy, arterial hypertension (>130 mmHg systolic and >85 mmHg diastolic) or ongoing pharmacological treatment, hyperglycemia (fasting >100 mg/dl) or specific drug therapy or previous diagnosis of type 2 diabetes mellitus (DM2). *(Alberti et al. 2009)*

The estimate of the waist-to-hip ratio (WHR) is also an accurate and valid parameter for the evaluation of abdominal obesity. According to the WHO data collection protocol, waist circumference should be measured at the midpoint between the lower edge of the last palpable ribs and the top of the iliac crest, using a tension-resistant tape measure. Hip circumference should be measured around the widest portion of the buttocks, with the tape measure parallel to the floor. (*WHO expert Consultation. World Health. 2008*)

Although there is no unanimity on what the appropriate cut-off is, the WHO in the 2000 obesity panel suggests that in Caucasian populations the WHR should be <1.0 in men and <0.85 in women. In line with this, waist circumference and WHR as indicators of central obesity are stronger independent risk factors for insulin resistance, type 2 diabetes, dyslipidemia and atherosclerosis than BMI. (*Guglielmi et al. 2018*)

Waist circumference and WHR allow us to distinguish three types of obesity (*Fig. 1.2*):

- Gynoid/peripheral: more frequently found in women, whose fat accumulates mainly at the subcutaneous level in the breast and hip region.
- Android/central type: more frequently found in men, in which fat is deposited at the abdominal, thoracic and cervical levels, around the internal organs. This type of obesity is associated with a worse prognosis for morbidity and mortality.
- Mixed: typology that combines the characteristics of both forms of obesity previously described.

Furthermore, adipose tissue is divided into two different types: white and brown adipose tissue.

The first is a full-fledged endocrine organ and takes part in numerous physiological processes such as: regulation of immune defenses, appetite, fertility and coagulation.

Brown adipose tissue, on the other hand, is useful in children to avoid strong heat loss at birth, while in adults it mainly has a thermoregulatory action. (*Baglioni et al. 2012*)

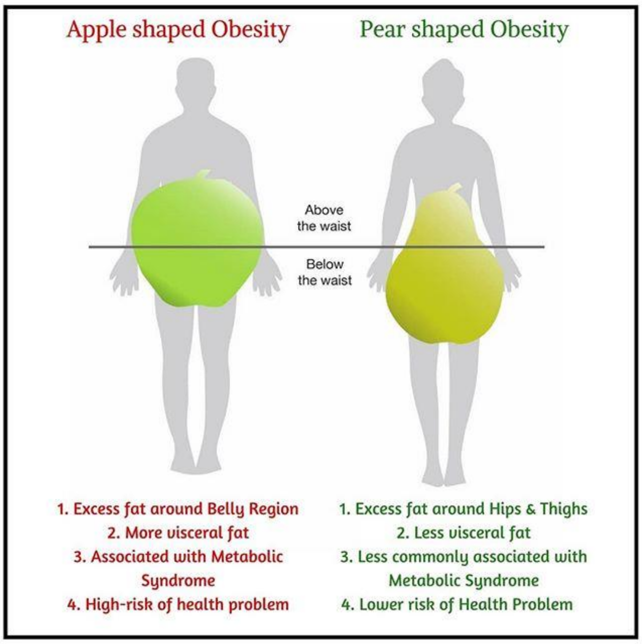

Figure 1.2: Types of Obesity
(https://komal.me/)

1.2 Obesity related comorbidities

Recently the WHO coined the term "Globesity" (global obesity) to indicate the spread of the problem on a planetary scale. Although not all obese people are affected by comorbidities, the increase in the prevalence of obesity is associated with the increase in related comorbidities. (*Guh et al. 2009*)

The latter depend in particular on BMI and the duration of obesity, and include (Fig. 1.3);

- cardiovascular comorbidities: arterial hypertension, accelerated atherosclerosis, coronary artery disease (CAD), left ventricular hypertrophy, cor pulmonale, obesity-associated cardiomyopathy, pulmonary hypertension and heart failure. Furthermore, it is believed that obesity, as well as being a marker, is also an independent risk factor for coronary atherosclerosis, heart failure and atrial fibrillation; (*Mandviwala et al. 2016*)
- surgical complications: increased surgical risk and post-operative complications such as

wound infection, pneumonia, deep vein thrombosis and pulmonary embolism; (*Quante et al. 2015*)

- skin comorbidities: dryness, itching, intertrigo (bacterial and/or fungal), acanthosis nigricans, hirsutism and increased risk of cellulite; (*Brown et al. 2013*)
- phlebological comorbidities: venous insufficiency, varicosities, venous and/or lymphatic edema of the lower limbs; (*Davies et al. 2017*)
- gastrointestinal complications: cholelithiasis, non-alcoholic steatohepatitis, gastroesophageal reflux; (*Guh et al. 2009*)
- metabolic comorbidities: glucose intolerance and type 2 diabetes mellitus, hyperuricemia and gout, metabolic syndrome and dyslipidemia (hypercholesterolemia, hypertriglyceridemia); *(Grundy et al. 2000*)
- perinatal obstetric comorbidities: gestational hypertension, fetal macrosomia and shoulder dystocia; (*Catalano et al. 2017*)
- orthopedic comorbidity: osteoarthritis, chronic low back pain, coxavar, epiphysiolysis, Blount's disease and Legg-calvè-Perthes disease; (*Davis 2012*)
- psychological comorbidities: social stigmatization and depression; (*Jantaratnotai et al. 2017*)
- respiratory comorbidities: obstructive sleep apnea syndrome (OSAS), Pickwickian syndrome (obesity hypoventilation syndrome), predisposition to infections and bronchial asthma; (*Mydin et al 2014*)
- reproductive complications: in women: menstrual irregularity, amenorrhea, anovulatory cycle, precocious puberty, fertility problems, hyperandrogenism and polycystic ovary syndrome (*Broughton et al. 2017*), hypogonadotropic hypogonadism in men; (*Lamm et al. 2016*)
- neurological comorbidities: stroke, idiopathic intracranial hypertension and meralgia paresthetica; (*O'Brien et al. 2017*)

- neoplasms: association with tumors of the endometrium, prostate, colon, breast, gallbladder, esophagus (recently demonstrated) and lung. (*Stroud et al. 2023; Guh et al. 2009*)

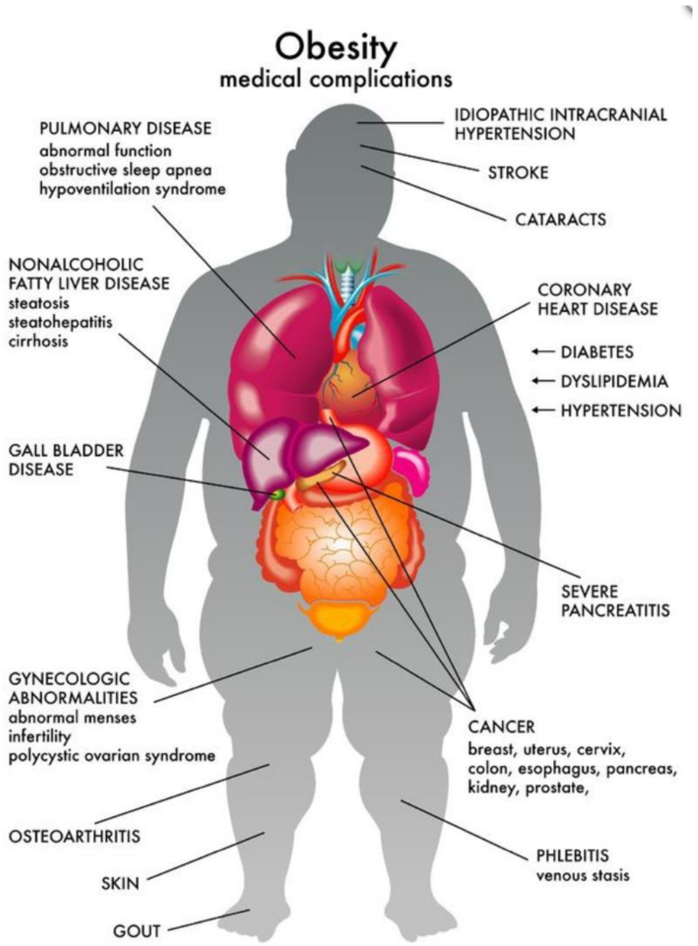

Figure 1.3: Obesity related Comorbidities
(https://www.endocrineweb.com/professional/obesity/obesity-cancer-evidence-grows-supporting-link)

According to WHO data in 2016, 28.4 million deaths worldwide (54% of total deaths) were attributable to ten conditions, of which six are direct consequences of obesity. (*Fontaine et al. 2009*) It has been shown that weight loss is essential for the prevention and treatment of obesity-related chronic diseases and that it is also associated with a reduction in mortality. Weight loss produces substantial benefits, reducing the number of years of life lost to comorbidities such as hypertension, hypercholesterolemia, type 2 diabetes, heart attack and stroke. (*Xiao et al. 2012*)

Weight loss in the short-term (6 weeks) and long-term (6-12 months) periods is associated with reductions in cardiovascular mortality for the following 4-5 years even in patients with pre-existing cardiovascular disease. (*Caterson et al. 2012*)

Also with regard to Non-Alcholic Fatty Liver Disease (NAFLD), it has been demonstrated that with a weight loss >7% there was an improvement in liver histology in 50% of patients.

The only side effect of weight loss, especially if it occurs rapidly, is the formation of gallstones with possible stones. (*Musso et al. 2012; D'Hondt et al. 2011*)

1.3 Type of procedures in Bariatric surgery

There are many bariatric surgery procedures performed in Italy and worldwide. The three most frequent type of BS are Sleeve gastrectomy (SG), Roux-en-Y Gastric Bypass (RYGB) and Single Omega Anastomosis Gastric Bypass (OAGB). (*Gentileschi et al. 2023*)

1.3.1 Sleeve Gastrectomy.

This is an irreversible operation which consists in the vertical resection of approximately ¾ of the stomach along the greater curvature with complete aspiration of the gastric fundus and creation of a gastric tubule with a capacity of 100-250ml. (*Fig. 1.4*)

This intervention has two components: a restrictive component and a functional component. The restrictive component is due to the neo-gastric pouch which, having a reduced volume, leads to a feeling of early satiety with less food intake. (*Sicob Guidelines 2023*)

As regards the functional component, it has been seen that the intervention causes advantageous changes in the hormones that regulate the sense of hunger-satiety and those used for glycemic control. These are ghrelin, peptide YY (PYY), pancreatic polypeptide (PP), and glucagon-like peptide 1 (GLP-1). (*Benaiges et al. 2015*)

Sleeve gastrectomy has been shown to achieve substantial weight loss and excess weight loss, often leading to significant improvements in obesity-related comorbidities. At 2 Year Post-Surgery: EWL can range from approximately 50% to 70%. (*Gagner et al. 2009*)

On the other hand weight regain after sleeve gastrectomy can vary widely from person to person and is influenced by various factors, including individual physiology, lifestyle choices, dietary habits, physical activity, and overall health. Long-term weight regain after sleeve gastrectomy is observed in a significant portion of patients. Studies have shown that up to 20-50% of patients may experience weight regain several years after the surgery. (*Peterli et al. 2018*)

Main complications of this surgery are: (*Sarkhosh et al. 2013; Salminen et al. 2018*)

• Hemorrhage (1-6%)

• Gastric fistula or Staple-Line leak (0,2 – 3,5%)

• Abscess (0.7%)

• Stenosis (0.2-4%)

• Nutritional deficits (20%)

• Gastroesophageal reflux disease (GERD/GERD)

Mortality rates associated with sleeve gastrectomy are typically very low. Studies and data often report mortality rates as a percentage or per 1,000 procedures. Mortality rates are usually well below 1% and often approach 0.1% or less. (*Salminen et al. 2018*)

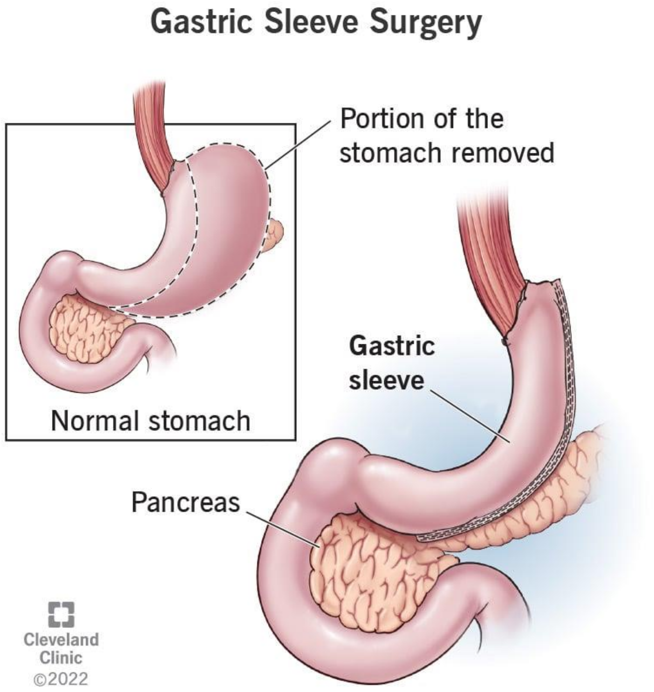

Figure 1.4: Sleeve Gastrectomy
(https://my.clevelandclinic.org/-/scassets/images/org/health/articles/22931-gastric-sleeve-surgery)

1.3.2 Gastric Bypass

Gastric by-pass is a mixed procedure with a restrictive, functional and hypoabsorptive component. The first phase of the operation is represented by the creation of a small neo-gastric pouch (20-30ml) at the level of the cardiac portion which is anastomosed with the small intestine. The second anastomosis is performed more distally and connects the loop through which the bolus will pass (alimentary loop) with the loop through which the gastric juices pass (biliopancreatic). (*Fig. 1.5*)

In this operation therefore, most of the stomach and duodenum are excluded from food transit. The weight loss is due both to the restrictive component of the intervention which leads to a reduction in food intake due to premature satiety and appetite and to the functional component which is a reduction in appetite and early satiety due to the release of some hormones by a section of the intestine that receives an undigested bolus and is not used to receiving it in this form. Finally, the malabsorptive component is due to the exclusion of a large part of the stomach, the duodenum and the first part of

the jejunum from food transit. There are some variations of this procedure but the most used are the Roux loop gastric bypass (RYGB) and the Mini Gastric By-pass (MGB) better defined as Gastric By-pass with single anastomosis (SAGB), with a single anastomosis (OAGB) or Ω loop . *(Fig. 1.6)*
The difference between the two methods consists in the size of the gastric pouch and the number of anastomoses. In RYGB the pouch is smaller and two anastomoses are made: a gastro-jejunal and a latero-lateral jejunal, constructing a food loop of 100-150 cm and a biliary-pancreatic loop of 50-75 cm. In OAGB the gastric pouch is longer (60 ml) and the single end-to-side anastomosis between the alimentary and biliodigestive tracts is at a distance from the duodenum, not entirely standardized, which normally corresponds to approximately 200 cm. This is a fundamental element, since in this way the distance of the gastric anastomosis from the ileo-cecal valve can vary from 600 to 200 cm, giving the operation a completely unpredictable hypo-absorbent connotation. (*Sicob Guidelines 2023*)

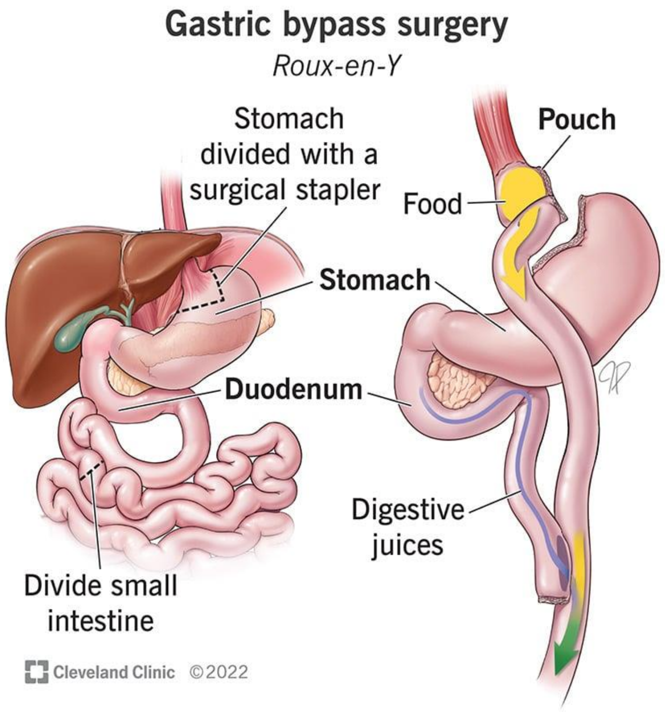

Figure 1.5: Roux en Y Gastric Bypass (RYGB)
(https://my.clevelandclinic.org/health/treatments/17157-gastric-bypass-surgery)

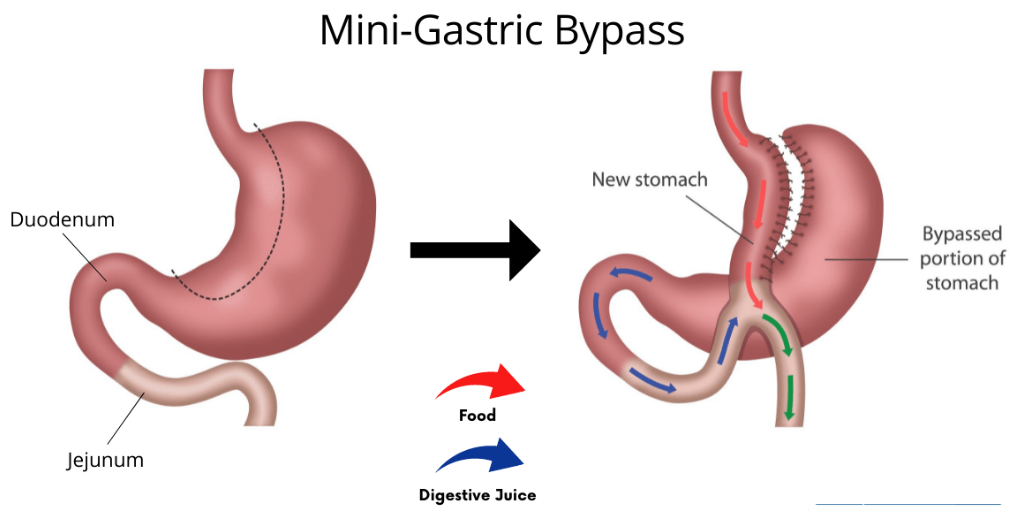

Figure 1.6: Mini-Gastric Bypass or Omega Gastric Bypass (OAGB)
(https://mexicobariatriccenter.com/wp-content/uploads/2022/01/Mini-Bypass-Procedure-1.png)

Numerous studies have shown that in addition to the previously mentioned mechanisms, another mechanism contributes to weight loss in both procedures: a change in the hormonal structure.

Among these, it was seen that ghrelin, a hormone that reduces appetite, begins to decrease two weeks after surgery. *(Beckman et al. 2012; Dimitriadis et al. 2017*)

A hormone whose levels increase post-intervention is glucose-dependent insulinotropic polypeptide (GIP) associated with an increase in insulin secretion following the ingestion of glucose with a meal. (*Dimitriadis et al. 2017*)

Alongside GIP, Glucon-Like Peptide type 1 (GLP-1) also increases following surgery. This hormone facilitates insulin secretion in a glucose-dependent manner, decreasing blood sugar levels. In addition to insulinotropic effects, GLP-1 has been associated with numerous regulatory and protective effects. Glucon-Like Peptide type 2 (GLP-2), produced by distal intestinal endocrine cells (L cells) and various neurons in the central nervous system, is co-secreted with GLP-1 following a meal. GLP-2 increases post-operatively, stabilizing weight loss and participating in the regulation of specific aspects of hunger-satiety. (*Cazzo et al. 2017*)

Peptide YY (PYY) is reduced after Gastric Bypass. Its main role appears to be central regulation of appetite with reduction of food intake. It also causes delay in gastric emptying, reduction in postprandial insulin production and alteration of cardiac motility. Insulin level, a hormone produced by the beta cells of the pancreas, regulates the metabolism of carbohydrates, fats and proteins, particularly promoting the absorption of glucose from the blood into adipose tissue, liver and skeletal muscle. It has been shown that after bypass the function of beta cells is increased with consequent improvement in glycemic control. (*Dimitriadis et al. 2017*)

The main complications of RYGB: (*Ma et al. 2015*)

• failure to seal (leak) of the anastomosis (0.7 - 4,5%)

• intestinal obstruction (0.5-5%)

• gastrointestinal haemorrhage (1%<)

• dumping syndrome (24.3%)

• internal hernia (3-16%)

• intussusception (0.1-1%)

• gallstones (22-71%)

• nutritional deficits, the most common of which are those concerning Vitamin B12, iron, Thiamine, Calcium. (*Malinowski et al. 2006*)

As regards the main complications of OAGB, bile reflux is added to those cited for RYGB. The reported incidence of bile reflux after OAGB ranges from approximately 0.3% to 10% in literature. (*Musella et al. 2017*)

1.4 Adiponectin (APN)

In recent years, the increasing occurrence of obesity has focused scientific research on the study of adipose tissue leading to the discovery that the white adipose tissue is able to secrete important molecules with endocrine, paracrine and autocrine meaning, known together as adipokines. Adipose tissue is today considered a proper endocrine organ. (*Harwood, 2012*) Actually, the molecules produced by the white adipose tissue significantly interfere with many kinds of metabolic activities (lipid and glucose metabolism) and are also involved in the regulation of the cardiovascular functions (blood pressure, homeostasis, angiogenesis), immune system and feeding behaviour. (*Kershaw et al. 2004*)

Adiponectin (APN) is the most represented adipokine in the blood with physiological levels in the range of 5-30 µL/mL, it is secreted by adipose tissue and constitutes approximately 0.1% of all serum proteins. (*Obata et al. 2013*) There are two types of adipose tissue in humans, white adipose tissue and brown adipose tissue. Adiponectin is mainly secreted by white adipose tissue which has endocrine activity. Other tissues such as the liver, muscle, placental and bone secrete a smaller amount. (*Guerre-Millo 2002*)

Adiponectin occurs in oligomers of different molecular weight: (*Kadowaki et al. 2005*)

- Low molecular weight (LMW)
- Medium molecular weight (MMW)
- High Molecular Weight (HMW)

The main biological activity includes: induction of fatty acid synthesis, facilitation of insulin uptake in the muscle and inhibition of hepatic gluconeogenesis. (*Cook et al. 2000*) In the literature it has been demonstrated that adiponectin plays a role in improving insulin- resistance by reducing intracellular adipose deposits through the oxidation of fatty acids with the activation of the peroxisome proliferator-activated receptor (PPAR), consequently preventing the increase in free fatty acids as an effect of a high lipid content diet. It also increases the expression of insulin receptor substrates in the liver and muscle. (*FIG. 1.7*) (*Berendoncks, A.M.et al. Eur. J. Prev. Cardiol. 2015; Schindler et al. 2017*)

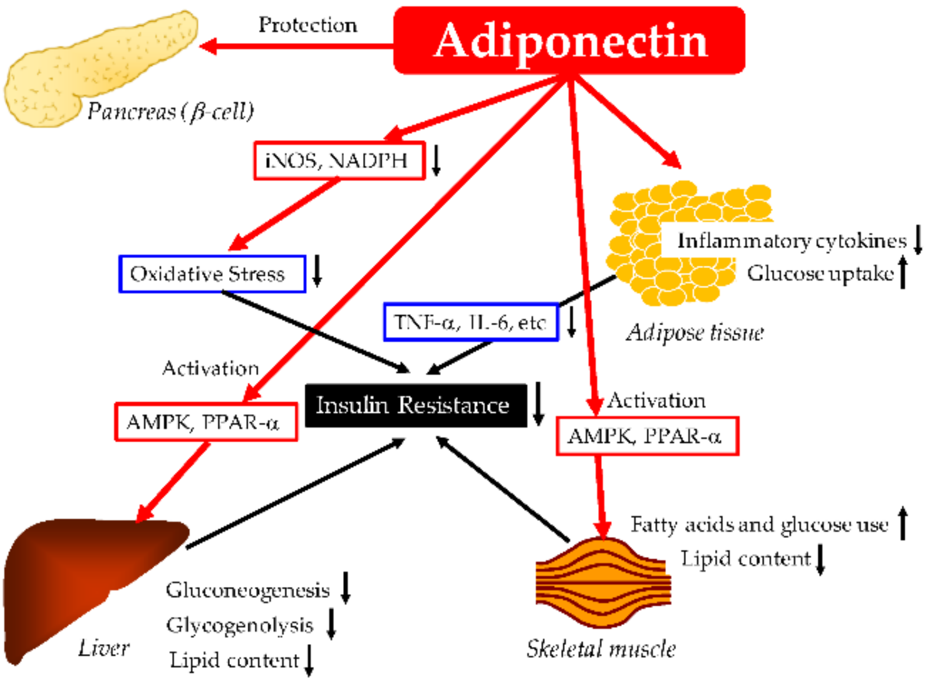

Figure 1.7 Adiponectin fuctions

Furthermore, adiponectin has shown antioxidant, anti-inflammatory and anti-atherosclerotic properties. (*Nur Aisiyah Widjaja et al. 2023*)

Many data in the literature report how adiponectin negatively correlates with anthropometric parameters such as body weight and BMI, and also with metabolic parameters such as blood sugar, total cholesterol and LDL cholesterol. Conversely APN correlates positively with HDL cholesterol. (*Nur Aisiyah Widjaja et al. 2023*)

However in a Swedish study by Herder et al. conducted on 1570 patients undergoing bariatric surgery in whom serum adiponectin was sampled, no relationship was highlighted in predicting outcomes after surgery. (*Herder et al. 2014*)

1.5 Aim of the thesis

The main objectives of BS are to promote a significant and sustainable weight loss to improve or resolve comorbidities and to promote a better quality of life with low rates of preoperative and long-term complications. However, weight loss is not homogeneous even with technical standardization of the surgery. This is why it can be crucial to identify a solid predictor of weight loss. The aim of our study is the identification of prognostic, anthropometric, hematological and biomolecular factors as predictors of outcome in patients undergoing bariatric surgery (RYGB, OAGB and Sleeve Gastrectomy). We collected data from a cohort of 130 subjects affected by severe obesity, who underwent bariatric surgery and were followed for additional 24 months. In this cohort we performed a clinical evaluation and pre-operative study tests on blood samples, as well as biomolecular analysis of subcutaneous and visceral adipose tissue and liver tissue specimens taken during the BS, with the ultimate goal of finding relationship between possible biomarkers i.e. TG level or adiponectin levels (VAT, SAT and serum) and medium and long-term prognosis.

Among the proposed outcomes, particular attention was paid to the success of the procedure understood as weight loss greater than 50% of excess weight or Excess Weight Loss (EWL), weight regain or insufficient weight loss, remission of comorbidities.

Chapter 2

MATERIAL AND METHODS

2.1 Patients, Study design and Ethical Approval

The study was performed on a series of patients suffering from severe obesity (Body Mass Index, BMI≥35 kg/m2 with comorbidities, or BMI≥40 kg/m2) referring to the Bariatric Surgery Unit at Santa Maria Nuova Hospital in Florence. A total of 130 patients were All patients enrolled and underwent laparoscopic bariatric surgery from September 2017 to September 2021 and were followed for subsequent 24-month observation after surgery.

From September 2018 to September 2021 a total of 130 patients were enrolled in our study. During 2020, recruitment activity was significantly affected and slowed down and even suspended for 18 months due to the interruption of elective surgical activity for non-neoplastic pathologies due to the COVID19 health emergency.

The study was approved by the Local Ethical Committee and Institutional Review Board (protocol number 83/13). All patients enrolled gave signed informed consent after receiving written and oral information on the study. Research has been performed in accordance with the Declaration of Helsinki. PThe manuscript follows the person first language guidelines for obesity was followed.

The choice of surgery and type of laparoscopic bariatric technique (Anastomosis Gastric Bypass, Roux Y Bypass, and Sleeve Gastrectomy) was made by a team of specialists composed by a surgeon, an endocrinologist and a dietitian, depending on the overall evaluation of the patient's history.

All patients underwent a pre-surgery baseline visit (T0) at hospital admission before surgery, taking all anthropometric measures, together with blood samples drawn for routine biochemical analysis.

At time of surgery, liver sample (LT) at III hepatic segment and two small AT samples (1 cm2) were taken from abdominal SAT and VAT, with no compromising of surgery. LT preserved in formalin was sent to the Anatomy Pathology Center for routine histological analysis, with a request for staging and grading of steatosis / steatohepatitis according to Brunt Score staging. (*Brunt et al. 2021*)

AT samples were snap frozen in liquid nitrogen and stored at -80°C until use for protein extraction and analysis. The follow-up evaluation was performed after 6, 12, 18 and 24 ±2 months from surgery. After surgery, patients were followed through clinical visits and haematological tests every 6-8 months at the bariatric clinics of the S. Maria Nuova Hospital in compliance with normal clinical practice for a minimum period of 2 years (patients operated between 2017 and 2020). Changes in terms of weight, BMI, and biohumoral values were recorded as well as the clinical status linked to the evolution of any comorbidities present at time of surgery (arterial hypertension, diabetes mellitus, sleep apnea, dyslipidemia, etc.).

In a subgroup of 43 patients, objects of a pilot study recently published, the levels of adiponectin (APN) were measured both in the VAT and SAT specimens samples taken (Vat and SAT) and in blood samplesthe circulating amount. . Some samples of subcutaneous and visceral adipose tissue were processed in the laboratory to obtain a primary cultures of adult stem cells according to a protocol optimized in the laboratory of Prof. M. Luconi (*Baglioni et al. 2009; Baglioni et al. 2010*), useful for experimentation and pathophysiological studies of adipose tissue.

2.2 Biochemical and Anthropometric Measurements

Anthropometric measures: Height, weight, waist and hip circumference were measured for each subject at baseline and at all the follow up time points; body mass index (BMI) was calculated according to the formula BMI (Kg/m2), EWL was calculated as: (Baseline Weight - Follow up Weight)/(Baseline Weight - Ideal Weight); Ideal Weight was considered for each patient as the weight corresponding to a BMI=25 Kg/m2. Waist circumference was measured between the lowest rib and iliac crest on bare skin. Participants were instructed to breathe in, and then out, and to hold their breath while measurement was made to the nearest .1 cm using a measuring tape. Hip circumference (HC) was determined at the maximum perimeter of the hips. For both WC and HC, the mean of two independent readings was used in analysis. Hip circumference and Ht were divided into WC measurements to derive WHR and WHtR indices.

Biochemical tests were performed on blood samples collected at baseline before surgery, after overnight fasting. Serum levels of fasting glucose, total cholesterol, HDL-cholesterol and triglycerides were measured using a colorimetric assay (Siemens Healthcare, Tarrytown, NY). LDL-cholesterol was calculated by Friedewald's formula for serum triglyceride levels < 400 mg/dL. Insulin was evaluated by an electrochemiluminescence immunoassay (ECLIA, Roche Diagnostics, Mannheim, Germany) on a Cobas E601 analyser (Roche Diagnostics). IR was estimated by the homeostasis model assessment of insulin resistance (HOMA-IR) index [(Insulin (pmol/L)×Fasting blood glucose (mg/dL)/22.5]; Min and Max blood pressure was measured at baseline.

Post-surgery complications were evaluated by the team of bariatric surgeons at each time point of the follow up, and classified as presence or absence.

2.3 Surgical technique

All interventions were performed by 4 bariatric surgeons each of them with experience of over 250 procedures in S. Maria Nuova Hospital in Florence, SICOB (Italian Society of Surgery of Obesity) Centre of excellence. All procedures were performed by laparoscopy with standard technique as previouslyprevious described by our SICOB Obesity Surgery Center. (*Soricelli et al.2017 ; Gentileschi et al. 2023*)

2.3.1 Roux-en-Y-gastric bypass (RYGB)

Roux-en-Y-gastric bypass (RYGB) includes 1) gastric pouch creation at 6 cm from the cardias using Linear stapler sized on 38 Fr bougie 2) creation of biliopancreatic limb of 75 cm from Treitz, 3) creation of gastrojejunostomy with 45 mm linear stapler 4) creation of jejunojejunostomy with 60 mm linear stapler.

2.3.2 Sleeve gastrectomy (SG)

Sleeve gastrectomy (SG) includes 1) preparation of gastric greater curvature and section of short gastric vessels 2) vertical partial gastrectomy creation using 60 mm Linear stapler from 6 cm by pylorus to para-cardial area sized on 38 Fr bougie.

2.3.3 Omega Anastomosis Gastric Bypass (OAGB)

Omega Anastomosis Gastric Bypass (OAGB) includes 1) gastric pouch creation starting at lesser curvature under the gastric "goose paw " using 60 mm Linear stapler sized on 38 Fr bougie 2) creation of biliopancreatic limb of 150 cm from Treitz, 3) creation of gastrojejunostomy with 45 mm linear stapler.

2.4 Adiponectin measurement

2.4.1 ELISA

APN was measured by a specific ELISA kit (Sandwich Enzyme-Linked Immunosorbent Assay for Quantitative Detection of Human Adiponectin Concentrations in Cell Culture Supernatants, Serum, Plasma, Tissue Homogenates, catalogue # MBS824844, My Biosource Inc, San Diego, CA, USA) in both serum and in protein extracts from VAT and SAT, according to the manufacturer's instructions. APN was evaluated in 100 µl of serum drawn before surgery, while for intra-tissue measurement, 100 µg of protein extract obtained by homogenizer in RIPA buffer (20 mM Tris pH 7.4, 150 mM NaCl, 0.2 mM EDTA, 0.5% Triton-100, supplemented with 100x phosphatase inhibitor and 100x protease inhibitor, Sigma-Aldrich) and clarified by centrifugation (6000 rpm 10 min 4°C), were analyzed. Sensitivity: the minimum detectable dose of Human APN is typically below 15 pg/ml; range of linearity: 62.5 pg/ml-4000 pg/ml.

2.4.2 Western Blot semiquantitative analysis

Frozen AT samples were homogenized and extracted in RIPA buffer. Thirty micrograms of extracted proteins were separated by 4-20% reducing SDS-PAGE (Stain-free precasted gels, BIO-RAD Labs). An internal standard (IS) made of equal amount of proteins extracted from 3 SAT and 3 VAT specimens for a total of 30 µg of proteins was applied in all SDS-PAGE as an internal anchor. Total protein loading for each lane was evaluated by Stain-free technique (BIO-RAD Labs) before protein transfer to PVDF membranes. Membranes were probed with anti-APN primary antibody (Vinci Biochem) followed by peroxidase-conjugated secondary IgG (13). Bound antibodies were revealed with ECL reagents (Immobilon, Merck Millipore). Image acquisition and densitometric analysis were performed with Quantity One software on a ChemiDoc XRS instrument (BIO-RAD Labs). All Western blots were repeated in at least three independent experiments. Semiquantitative APN expression was evaluated from Western blot after normalization of each lane for total protein loading and referred to IS expression taken as 1.

2.5 Statistical Analysis

Data were expressed as mean±SD or median [interquartile range, IQR]. Nonparametric U Mann-Whitney's test was used for comparisons of two sets of paired data and for independent data. Also Student's t test was used for comparison of parametric continuous data and Chi2 for non continuous variables. A $P<0.05$ value was used for statistical significance. Linear regression was evaluated by Pearson's or Sperman's tests Stepwise at univariate analysis or multiple linear regression was applied for multivariate analysis. Receiver operating characteristic (ROC) curve analysis for accuracy determination and all the other statistical analyses were performed on SPSS 28.0 for Windows (Chicago, USA).

Chapter 3.

RESULTS

3.1 Differences in clinical profiles between sexes in patients undergoing bariatric surgery

A total of 130 patients affected by severe obesity and undergoing bariatric surgery at Santa Maria Nuova Hospital in Florence between 2017 and 2021 were enrolled in the study. Patients' characteristics at baseline before surgery are indicated in both the entire cohort and after stratification for sex (*Tab. 3.1*).

	TOT		F		M		
PARAMETER (n.)	**MEAN**	**d.s.**	**MEAN**	**d.s.**	**MEAN**	**d.s.**	***P***
WEIGHT (130)	117,63	22,56	112,071	18,1852	140,960	24,5040	***0,000***
BMI (130)	43,96	6,51	43,289	6,0109	46,784	7,8186	***0,015***
AGE (129)	47,98	10,55	47,548	10,8510	49,800	9,1378	*n.s*
WAIST (120)	117,06	15,18	113,888	13,4646	131,182	14,5623	***0,000***
HIP (118)	131,70	12,56	131,844	11,3836	131,091	17,1045	*n.s*
W/H (126)	0,89	0,12	0,8682	0,09512	1,0135	0,16557	***0,000***
GGT (69)	33,28	23,84	31,000	24,6155	40,235	20,4100	*n.s*
AST/GOT (89)	23,06	9,34	21,929	9,4889	27,211	7,6418	***0,028***
ALT/GPT (89)	27,73	15,07	24,629	12,5861	39,158	18,0901	***0,000***
GLYCEMIA (47)	111,45	35,20	108,471	29,4271	119,231	47,7147	*n.s*
HbA1c (46)	39,41	6,53	39,000	6,5670	40,462	6,5653	*n.s*
INSULIN (46)	20,26	18,28	16,970	9,7066	28,615	29,9403	***0,051***
HOMA INDEX2.5 (39)	6,00	6,25	4,582	3,1474	9,600	10,0806	***0,022***
CHOLESTEROL (49)	211,92	52,19	214,278	54,7444	205,385	45,7102	*n.s*
HDL (50)	51,32	12,57	52,865	12,6297	46,923	11,7577	*n.s*
LDL (48)	131,90	45,60	133,823	45,3604	126,708	47,6983	*n.s*
TRIGLYCERIDES (49)	170,22	116,31	164,583	106,9225	185,846	142,8413	*n.s*
Pres min (122)	86,93	10,70	86,525	10,3553	88,696	12,1634	*n.s*
Pres max (122)	140,12	16,77	139,667	16,0979	142,087	19,6837	*n.s*

PARAMETER		N.	%	N.	%	N.	%	P
SEX	F	105	80,8					
	M	25	19,2					
	Total	130	100,0					
TYPE OF SURGERY	OAGB	60	46,2	44	41,9	16	64,0	
	RYGB	41	31,5	36	34,3	5	20,0	
	SG	27	20,8	24	22,9	3	12,0	*n.s*
	other	2	1,5	1	1,0	1	4,0	
	Total	130	100,0	105	100,0	25	100,0	
OSAS	NO	109	83,8	94	89,5	15	60,0	
	YES	21	16,2	11	10,5	10	40,0	***0,001***
	Total	130	100,0	105	100,0	25	100,0	
STEATOSIS	NO	12	9,2	10	9,5	10	9,5	
	Grade I	34	26,2	29	27,6	29	27,6	
	Grade II	36	27,7	32	30,5	32	30,5	***0,035***
	Grade III	17	13,1	15	14,3	15	14,3	
	Total	99	76,2	86	81,9	86	81,9	
FIBROSIS	NO	18	13,8	17	16,2	1	4,0	
	Stage I	18	13,8	17	16,2	1	4,0	
	Stage II	34	26,2	31	29,5	3	12,0	
	Stage III	11	8,5	10	9,5	1	4,0	***0,037***
	Stage IV	2	1,5	2	1,9	6	24,0	
	Stage V	16	12,3	10	9,5	12	48,0	
	Totale	99	76,2	87	82,9			
	Mancante	31	23,8					
NASH	NO	26	20,0	21	20,0	5	20,0	
	Nash I	41	31,5	37	35,2	4	16,0	
	Nash II	8	6,2	8	7,6	0	0	*n.s*
	Total	75	57,7	66	62,9	9	36,0	
	Missing	55	42,3					
HYPERTENSION	NO	60	46,2	74	70,5	16	84	
	YES	63	48,5	31	29,5	9	36	
	Total	123	94,6	105	100	25	100	*n.s*
	Missing	7	5,4					
DIABETES	NO	110	84,6	88	67,69	22	16,92	
	YES	20	15,4	17	32,31	3	2,3	*n.s*
	Total	130	100,0	105		25		

Table 3.1 Baseline data Continuous and Discrete Variables (p= 0,05 for statistical significance)

Mean BMI was 43,3 Kg/m2 in female subjects and 46,8Kg/m2 in male subjects with a mean Waist /Hip Ratio of 0,87 and 0,95 respectively. Among the 105 females, 32.3% had T2D and 10,5% were affected by OSAS, while among the 25 males, 3% had T2D and 40% were diagnosed with OSAS and used cPAP during the night. One Anastomosis gastric bypass (OAGB) surgery technique was performed in 41,9% and 64%, Roux Y Gastric Bypass (RYGB) in 34.3% and 20%, and Sleeve Gastrectomy (SG) in 22.9% and 12% of the females and males, respectively. Post-surgery major complication, according to DindoClavien classification > grade III (*Clavien et al. 2009*) was a single case of severe surgery-related bleeding in a woman (0,76%).

Statistical analysis of data showed significantly better weight baseline parameters in females compared to males, and higher "pear-shape" obesity distribution in females than in males as demonstrated by W/H ratio and waist measurement. Another significant difference were lower levels of circulating insulin and a better hepatic profile (lower AST/GOT and ALT/GPT levels), in females compared to males (Tab. 3.1). No further differences at baseline between sexes were found when stratifying for the type of surgery (not shown).

3.2 Correlation between EWL and IWL and clinical data stratified between sexes

When looking at the excess weight loss (EWL) as a clinical endpoint of the effects of surgery, EWL was found to be higher in females at any time points after bariatric surgery, corresponding to significantly lower BMIs reached at 12, 18 and 24 months of follow up. (Tab.3.2).

	F		M		
PARAMETER (n.)	**MEAN**	**d.s.**	**MEAN**	**d.s.**	***P***
EWL 6m	69,547	20,7973	66,027	29,5298	*n.s*
EWL 12m	80,869	22,6910	70,677	25,7292	***0,051***
EWL 18m	86,739	22,6432	72,659	27,3240	***0,08***
EWL 24m	86,437	23,1554	72,638	32,8901	***0,016***
BMI 6m	31,207	4,9948	33,146	7,6820	*n.s*
BMI 12m	29,217	4,8126	32,457	6,7674	***0,03***
BMI 18m	28,066	4,6171	31,834	6,5296	***0,001***
BMI 24m	28,033	4,6825	31,706	6,9351	***0,02***

Table 3.2 Mean±SD of EWL and BMI values in the two sexes at different times of the follow up. (F= Female; M= Male). Female subjects reach significantly higher EWL at 12, 18 and 24 months. P values calculated by Student's test are indicated, ns= not significant.

EWL was always higher in females compared to males, with a statistically significant difference starting form 12 months (Tab. 3.2). No difference were found in EWL at 6 and 24 months between sexes when stratified by type of BS (not shown).

A linear correlation was found between basal clinical variables and EWL at 6, 12, 18 and 24 months, when stratified by sexes (Tab.3.3).

	EWL 6m				EWL 12m				EWL 18m				EWL 24m			
parameters	sex F		sex M		sex F		sex M		sex F		sex M		sex F		sex M	
	R	P	R	P	R	P	R	P	R	P	R	P	R	P	R	P
BMI	**-,517****	**0,000**	-,635**	*0,001*	**-,533****	**0,000**	-,520**	*0,008*	**-,476****	**0,000**	-,431*	*0,032*	**-,413****	**0,000**	-0,304	*0,140*
WAIST	**-,355****	**0,000**	-0,271	*0,223*	**-,440****	**0,000**	-0,291	*0,189*	**-,479****	**0,000**	-0,233	*0,298*	**-,453****	**0,000**	-0,119	*0,597*
W/H ratio	**-,197***	**0,047**	-0,313	*0,146*	**-,243***	**0,013**	-0,410	*0,052*	**-,279****	**0,004**	-0,336	*0,117*	**-,270****	**0,006**	-0,297	*0,168*
HbAc1	0,169	*0,349*	-0,305	*0,311*	-0,122	*0,498*	-0,396	*0,180*	**-0,339**	**0,054**	-0,383	*0,196*	**-,348***	**0,047**	-0,202	*0,509*
TG	-0,009	*0,958*	0,176	*0,566*	-0,193	*0,259*	0,174	*0,569*	**-,341***	**0,042**	0,119	*0,698*	**-,437****	**0,008**	0,005	*0,986*
OSAS (y/n)	-0,155	*0,115*	-0,155	*0,115*	**-,206***	**0,035**	**-,206***	**0,035**	**-,204***	**0,037**	**-,204***	**0,037**	**-,204***	**0,038**	**-,204***	**0,038**
FIBROSIS (y/n)	0,021	*0,847*	0,021	*0,847*	-0,174	*0,107*	-0,174	*0,107*	**-,224***	**0,037**	**-,224***	**0,037**	**-,249***	**0,021**	**-,249***	**0,021**
NASH (y/n)	**-,259***	**0,036**	**-,259***	**0,036**	**-,298***	**0,015**	**-,298***	**0,015**	**-,252***	**0,041**	**-,252***	**0,041**	**-,280***	**0,024**	**-,280***	**0,024**

Table 3.3 Univariate analysis: correlation between clinical parameters, EWLs and sexes.

When the association between EWL (short term = 6 months and long term=24 months) and the variables found to be significant at univariate was further subjected to multivariate analysis to identified the best predictors, only the correlations in data in female patients resulted to be statistically significance (Tab.3.4).

Factor	EWL 6M				EWL 24M			
	univariate		multivariate		univariate		multivariate	
	r	p	β	p	r	p	β	p
W/H (n=103)	**-0.197**	**0.047**	-0.260	0.203	**-0.270**	**0.006**	-0.251	0.188
BMI (n=105)	**-0.517**	**0.000**	**-0.420**	**0.022**	**-0.413**	**0.000**	*-0.300*	*0.074*
HbA1c (n=33)	0.349	0.169	**0.582**	**0.032**	**-0.348**	**0.047**	-0.022	0.927
Glycaemia (n=70)	-0.015	0.932	-0.312	0.317	**-0.333**	**0.050**	-0.001	0.998
Triglycerides (n=36)	-0.009	0.958	-0.048	0.823	**-0.437**	**0.008**	**-0.443**	**0.036**
OSAS (n=104)	-0.155	0.115	*-0.215*	*0.075*	**-0.204**	**0.038**	**-0.251**	**0.040**
Fibrosis N/Y (n=87)	0.021	0.847	0.186	0.178	**-0.249**	**0.021**	-0.084	0.542
NASH N/Y/+NAFLD (n=66)	**-0.297**	**0.015**	**-0.364**	**0.009**	**-0.282**	**0.023**	-0.210	0.129

Table 3.4 Linear regression analysis of factors predicting rapid and long term EWL in female subjects. Among factors associated with EWL in the short term (6 months) after bariatric surgery, only BMI and HbA1c and NASH maintain statistical significance, while in the long run (24 months), only triglyceride levels and the OSAS.

In particular, when considering short (6 months) and long terms (24 months) for EWL, iin the short term (6 months) after bariatric surgery, only BMI and HbA1c and NASH maintain statistical significance, while in the long run (24 months), only TG levels and OSAS.

No significant WR (Weight regain) was observed in our cohort even at two years from surgery. Insufficient weight loss (IWL), defined as WL<50% EWL (*El Ansari et al 2021*), at 24 months has a significantly higher frequency in males compared to females (24% vs 8.7%, p=0.030). Interestingly, in females, there is a positive correlation between the presence of NASH and IWL (r=0.263, p=0.033, n=66), while in males, LDL and cholesterol levels can significantly predict IWL with r=0.602, p=0.029, n=13 and r=0.579, p=0.038, n=13), respectively.

The type of surgery significantly affects IWL (p=0.000), being RGYB the most successful with only 2.4% of IWL, while IWL frequency was 14,70% and 11,10% after OAGB and SG, respectively. Insufficient weight loss at 24 months has a significantly higher frequency in males compared to females (24% vs 8.7%, p=0.030). Interestingly, in females, there is a positive correlation between the

presence of NASH and IWL (r=0.263, p=0.033, n=66), while in males, LDL and cholesterol levels can significantly predict IWL with r=0.602, p=0.029, n=13 and r=0.579, p=0.038, n=13), respectively.

3.3 Correlation surgery and comorbidities in the follow up.

Among comorbidites considered in our cohort, T2D, hypertension, cholesterolemia, psychiatric disorders, and gastroesophageal reflux were considered. We evaluate the effects of the type of surgery as well as the correlation with these pathologies and any other parameters so far analyzed. For each pathology a score of 0, 1, 2 was arbitrarily assigned, if at follow-up of 24 months the number of drugs decreased (remission or

Furthermore only with regard to female group of patients, OSAS has been shown to be a significant negative predictor of clinical remission (evaluated as interruption/reduction of pharmacological therapy) of Diabetes Mellitus (p 0,005) and Hypertension (p 0,038) and Hypercolesterolemia (p 0,003) after BS in long term follow up. amelioration of the pathology), remained stable (no alterations) or increased (worsening).

In *Fig. 3.1* it can be noted that RYGB is always associated with the highest rate of comorbidity amelioration, assessed by a reduction of the specific pharmacological treatments, while no worsening is reported for any comorbidity, except for psychotic disease, for which the 3 surgeries performed very similarly. SG performed negatively for GER, with a worsening or novel presentation in 75% of the patients and no subjects with amelioration, compared to OAG, for which the percentage of worsening was similar (75%). For HPT, only OAG was associated with a worsening of symptoms, while only SG was associated with a worsening of T2D.

Worsening of the pathologies was always contained below 30% of the population, except for CHOL and GER where only 25% of the patients did not worsen.

Statistical differences were not found in comorbidities remission stratifying by type of BS except for Psychiatric disorder and Gastroesophageal reflux (GER). (*Fig. 3.1*)

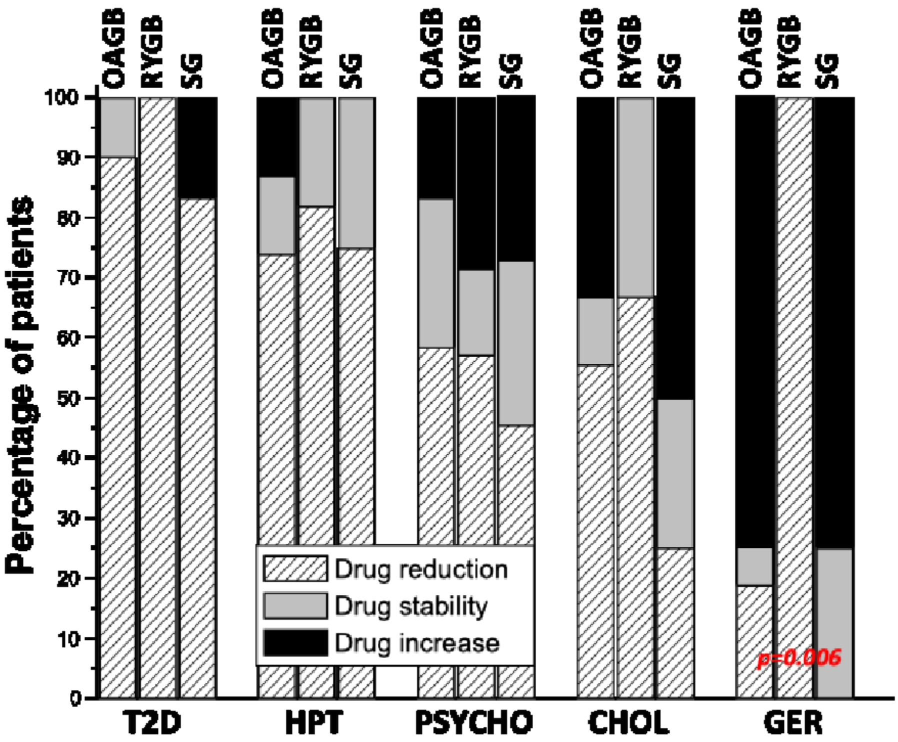

Figure 3.1: Association between the type of bariatric surgery and patients' comorbidities at 24 months of follow up. Frequency of the indicated comorbidities (T2D: type 2 diabetes, HPT: hypertension; PSYCHO: Psychotic disorder, CHOL: cholesterolemia, GER: gastroesophageal reflux) in the cohort of 130 bariatric patients) is shown as % bar graph for all the three types of bariatric surgery applied (OAGB: Anastomosis Gastric Bypass: RYGB: Roux Y Bypass, and SG: Sleeve Gastrectomy). Reduction, stability and increase in the pharmacological treatment specific for each comorbidity is indicated. Statistically significant differences between the results of the three types of surgery are detectable only for GER.

It can be noted that RYGB is always associated with the highest rate of comorbidity amelioration, assessed by a reduction of the specific pharmacological treatments, while no worsening is reported for any comorbidity, except for psychotic disease, for which the 3 surgeries performed very similarly. For HPT, only OAGB was associated with a worsening of symptoms, while only SG was associated with a worsening of T2D. Worsening of the pathologies was always contained below 30% of the population, except for CHOL and GERD where only 25% of the patients did not worsen. In fact SG performed negatively for GERD, with a worsening or novel presentation in 75% of the patients and no subjects with amelioration, compared to OAGB, for which the percentage of worsening was similar (75%). None of 42 patients who underwent RYGB developed GERD at 24 months even those with RGE therapy at time of surgery that had complete remission.

Of 61 OAGB patients we found in 4 of them with previous diagnosis of RGE a complete remission of symptoms at 24 months but in 13 patients they were highlighted "*deNovo*" RGE symptoms with incrementations of pharmacological therapy.

3.4 Differences in the blood and adipose tissue levels of APN between sexes in patients affected by severe obesity.

APN levels were measured only in 43 pts of the enrolled patients before surgery in paired samples taken at surgery from abdominal SAT and VAT and compared with serum levels of APN, all evaluated with the same ELISA technique (*Tab.3.5*).

	TOT	F (n=24)	M (n=17)	p
APN VAT (μg/mg)	3.5[2.5-5.1]	3.2[2.4-5.1]	3.6[2.5-5.2]	.771
APN SAT (μg/mg)	3.8[2.3-5.2]	3.6[1.9-5.7]	3.9[2.5-5.1]	.696
APN serum (μg/ml)	8.7[5.7-12.5]	9.8[6.7-13.8]	6.3[3.9-11.2]	**.045**
EWL 6m (%)	58[47-70]	63[55-76]	45[33-57]	**.004**
EWL12m (%)	73[63-83]	78[69-85]	63[52-77]	**.003**
EWL 18m (%)	83[72-90]	84[80-94]	74[58-85]	**.036**
EWL 24m (%)	82[72-93]	86[79-97]	73[52-90]	.126
BMI 6m (Kg/m^2)	33[30-37]	32[30-34]	35[34-41]	**.007**
BMI 12m (Kg/m^2)	31[28-33]	29[28-31]	34[31-37]	**.003**
BMI 18m (Kg/m^2)	29[27-31]	28[26-30]	31[29-33]	.080
BMI 24m (Kg/m^2)	28[26-31]	28[26-30]	30[28-34]	.090

Table 3.5. Adiponectin levels and weight data. Data for the entire cohort (TOT) and after sex stratification (F, M) are reported as the median [IQR] values for adiponectin (APN) levels evaluated by ELISA in visceral (VAT) and subcutaneous (SAT) AT and in serum, as well as for EWL and BMI at 6, 12, 18 and 24 months of follow up after bariatric surgery. Statistically significant p values <0.05 between distributions in females (F) and males (M) calculated with U Mann-Whitney's test are indicated in bold Italics.

Circulating APN levels were confirmed to be higher in females, while to our surprise, APN content in abdominal SAT and VAT was lower than in the male sex, though the difference did not reach any statistical significance (*Tab.3.5*).

Differences between sexes and fat depots in APN local production in AT of patients was further confirmed by applying a semiquantitative Western blot analysis of total protein extracts from SAT and VAT specimens (*Fig. 3.2a*).

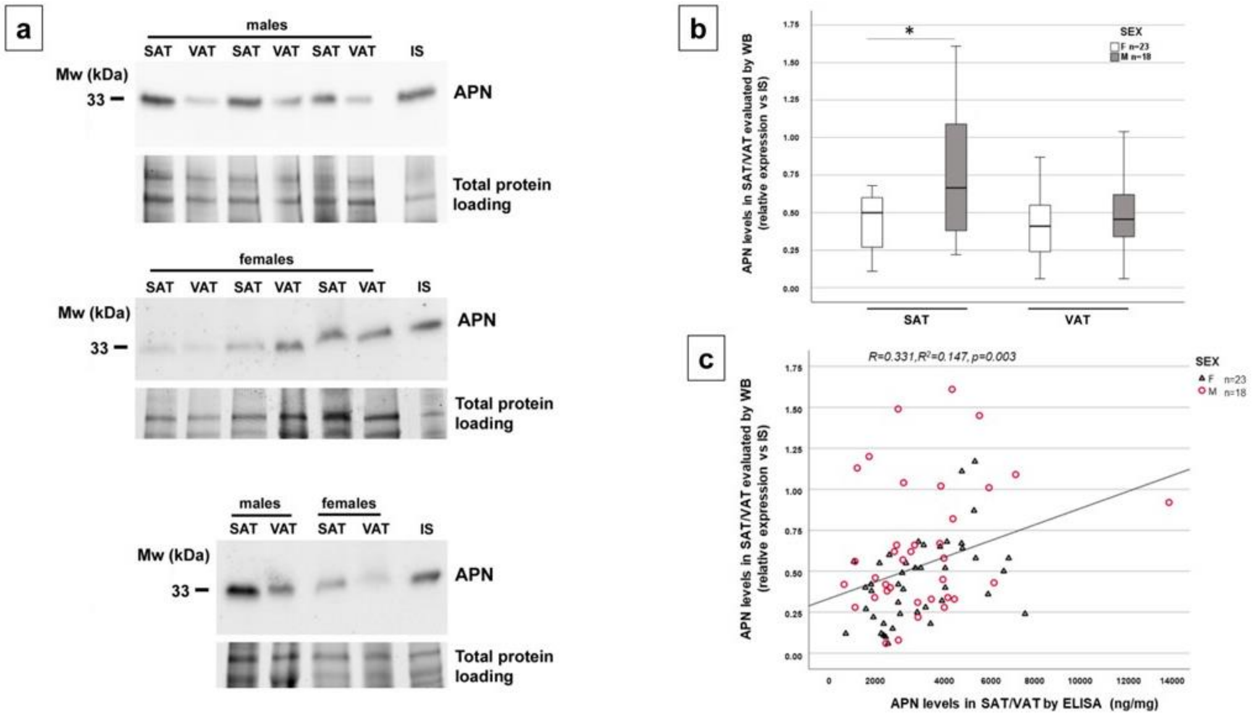

Figure. 3.2 APN protein expression in abdominal AT of patients with severe obesity: sex and depot differences. (a) Representative Western blot analysis of total protein extracts from SAT and VAT specimens of males and females patients with severe obesity shows a single band at the expected molecular weight, as revealed by the specific anti-APN antibody. An internal standard (IS) was run in all gels to enable semiquantitative relative expression of APN band intensity. Total protein loading for each lane as evaluated by Stain-free technique applied to Stain-free precasted gels (Biorad Labs). Molecular weight markers (MW in kDa) are indicated to left of the blot. (b) Box charts show the median[IQR] of semiquantitative APN levels, as evaluated in SAT and VAT protein samples evaluated by Western blot after normalization of each lane for total protein loading and referred to IS expression taken as 1. (c) Positive linear correlation between APN expression in AT (SAT and VAT) evaluated by relative semiquantitative WB analysis and by ELISA technique; females (triangle, F) and males (circle, M) are indicated.

APN relative expression compared to an internal standard (IS) of AT was confirmed to be higher in male vs female SAT, while the relative expression in VAT was lower and uniform among sexes

(Fig.3.2 a,b). A statistically significant correlation was found between APN in VAT and SAT evaluated by semiquantitative WB and quantitative ELISA (Fig.3.2 c). Contrary to what was previously reported in the group of 130 patients, the statistically significance of the differences in terms of EWL and consequent BMI between sexes was lost at the second year in this small subgroup of 43 patients.

APN levels in SAT, VAT and serum were not correlated with T2D or IR in either males and females.

3.5 VAT APN can predict EWL reached with bariatric surgery in women but not in men with severe obesity

In the subgroup of 43 pts a positive association was evident between baseline VAT APN and EWL only in females, which reached a statistical significance starting from 12 months after surgery and was also maintained at 2 years (*Fig. 3.3 a-d*).

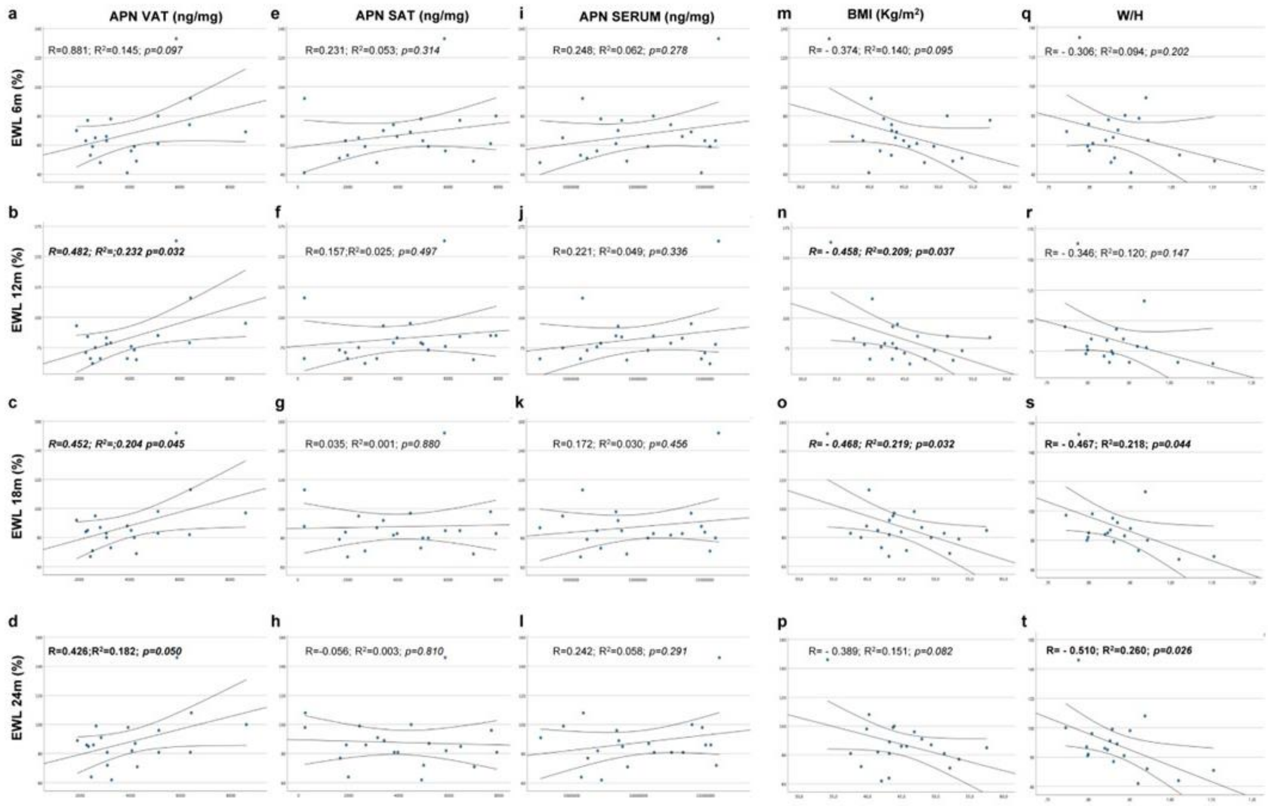

Figure 3.3 EWL association with AT (SAT and VAT) or circulating APN as well as with BMI or W/H in female patients with severe obesity. For each graph, linear interpolation curves and the 25-75th CI are depicted; R, R^2, and p values are reported; statistically significant p values are indicated in bold Italics; n=20 females where all EWL data were available.

On the contrary, no significant correlation was present for SAT (*Fig. 3.3 e-h*) or blood *(Fig. 3.3 i-l*) APN. Baseline BMI displayed a negative correlation with EWL, which was significant only at month 12 and 18 from surgery, and once again only when considering the female sex (*Fig. 3.3 m-p*). No correlation was found between age and APN levels or EWL, either in the whole cohort or in females, thus excluding any putative association with menopause.

Rather than BMI, a more penetrant indicator of fat excess distributed between visceral abdominal and subcutaneous femoral/gluteal AT, is represented by the waist/hip ratio, which indicates a healthier pear-shaped or a metabolically-risky apple-shaped phenotype, defined as below or greater/equal than the sex-related cut-off (respectively 0.8 in F and 0.95 in M). In our series, W/H correlation was significant only with circulating APN levels, r=-0.442, p=0.005. W/H was significantly correlated with EWL in females only and only starting from 12 months after surgery sex only (*Fig. 3.3 q-t*). The apple-shaped distribution was more frequent in females than males (77% vs 69%). APN in VAT was significantly higher in pear-shaped than in apple-shaped patients (p=0.007), and in females (p=0.019) compared to males (*Tab.3.6*).

	TOT (n=41)		**F (n=24)**		**M (n=17)**	
	PEAR	APPLE	PEAR W/H<0.8	APPLE W/H≥0.8	PEAR W/H<0.95	APPLE W/H≥0.95
APN VAT (μg/mg)	**5.4[4.2-6.5]*** **	**3.2[2.3-4.8**]	**4.1[5.9-7.5]***	**3.0[3.3-4.6]**	5.3[2.9-6.1]	3.3[2.1-4.9]
APN SAT (μg/mg)	4.9[3.4-5.4]	3.2[1.9-6.2]	5.2[4.2-5.9]	3.2[1.2-5.7]	4.9[1.8-5.0]	4.5[2.6-7.0]
APN serum (μg/ml)	**11.4[8.2-13.2]****	**6.4[4.6-10.9]**	12.5[8.8-15.0]	**8.5[6.6-11.1]**	11.1[6.1-12.2]	**5.2[3.8-6.3]§**

Table 3.6 APN distribution according to pear- or apple- shape in patients with severe obesity. Median[IQR] APN in VAT, SAT and circulating levels evaluated by ELISA are reported in the entire series of patients or are stratified according to female (F) and male (M) sex. U Mann-Whitney's test: *p=0.037, **p=0.019, and ***p=0.007 pear- vs apple-shape in the same sex; § p=0.019 F vs M.

In order to define the best threshold for VAT APN able to predict EWL after surgery, patients were stratified in two classes with VAT APN levels higher/equal or lower than the cut-off value, corresponding to the upper quartile (≥75th percentile) of the APN distribution in VAT samples, and identified by ROC curve analysis. In females, baseline VAT APN, but not baseline BMI, retained a statistically significant correlation with EWL at any time points between 6 and 24 months from surgery, both at univariate and multivariate analysis *(Tab.3.7)*.

UNIVARIATE	EWL 6m	EWL 12m	EWL 18m	EWL 24m
VAT APN ≥75th percentile vs <75th percentile	***r=0.594 p=0.005***	***r=0.621 p=0.003***	***r=0.585 p=0.005***	***r=0.521 p=0.015***
BMI	r= - 0.374 p=0.095	***r= - 0.458 p=0.037***	***r= - 0.468 p=0.032***	r= - 0.389 p=0.082
MULTIVARIATE	**EWL 6m**	**EWL 12m**	**EWL 18m**	**EWL 24m**
VAT APN ≥75th percentile vs <75th percentile	***β=0.545 p=0.007***	***β=0.558 p=0.004***	***β=0.519 p=0.008***	***β=0.467 p=0.025***
BMI	β= - 0.278 p=0.142	***β= - 0.360 p=0.045***	***β= - 0.378 p=0.042***	β= - 0.307 p=0.126

Table 3.7. Baseline visceral adiponectin is a better predictor than BMI in female morbidly obese patients of the EWL after bariatric surgery. Baseline VAT adiponectin (VAT APN cut off=5.1 µg/mg corresponding to the 75th percentile of distribution) evaluated by ELISA in female bariatric patients retains a statistical significant correlation with EWL at any time points between 6 and 24 months from surgery, both at univariate and multivariate analysis with the baseline BMI as confounding factor. Regression analysis was performed with the forward selection in the model with data from the No statistical significant correlation was found for male obese patients; n= 21 female patients; r, β and p are indicated. Statistically significant p values are indicated in bold Italics.

No correlation was found for SAT APN in females or, in general, in the male sex, or with W/H pear-vs apple-shaped classes (not shown). EWL was significantly higher in the upper quartile of VAT APN compared to lower levels at any time point from surgery in females, but not in males (Fig. 3.4 a). The type of bariatric surgery did not influence EWL percentage in either sexes (not shown). Finally, limited to the female patients, ROC analysis indicated the best cut-off values for VAT APN to predict 80% EWL at 6 months from surgery (98% accuracy, 100% sensitivity, 82% specificity, p=0.034, Fig.3.4 c) and 95% EWL at 12 months from surgery (98% accuracy, 100% sensitivity, 94% specificity, p=0.010, Fig.3.4 d). This analysis was not significant for male patients (not shown).

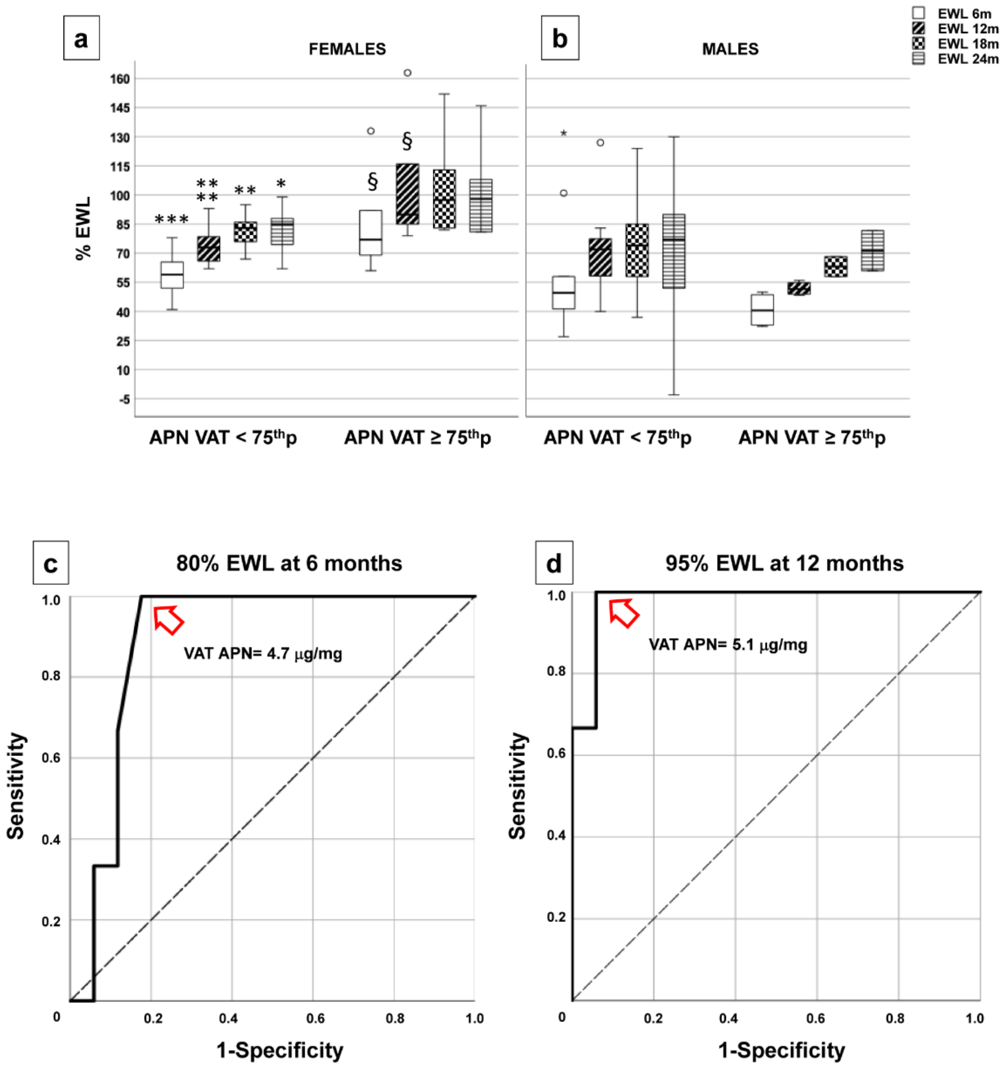

Figure 3.4: High VAT adiponectin levels are associated with EWL prediction in female severe obesity. Distribution of EWL in female and male patients: female (n=24, panel a) and male (n=19, panel b) patients were stratified in two classes according to the VAT APN cut off=5.1 □g/mg, corresponding to the 75th percentile of APN distribution. EWL Median[IQR] values at the different time intervals of follow up are indicated by box charts. Statistical analysis performed with U Mann-Whitney test: *p<0.05, **p<0.01, ***p<0.005, ****p<0.001 between VAT APN upper (□5.1 □g/mg) and lower (<5.1 □g/mg) classes; § p<0.010 between females (F) and males (M). Receiver operating characteristic (ROC) curve analysis for defining the best cut-off value of VAT APN predictive of the EWL in female patients: ROC curve analysis shown for APN concentration in VAT of 21 female patients out of 24 who had a complete follow up, and evaluated by ELISA, indicate that a cut-off of VAT APN of 4.7 □g/mg was able to predict 80% EWL at 6 months from surgery (89% accuracy, 100% sensitivity, 87% specificity, p=0.034), (panel c), while a cut-off of 5.1 □g/ml was able to predict 95% EWL at 12 months from surgery (98% accuracy, 100% sensitivity, 94% specificity, p=0.010), (panel d).

Chapter 4

DISCUSSION

In this study, when we look at the entire cohort of patients recruited (130 pts), the EWL was found to be higher in females at any time points after bariatric surgery, corresponding to significantly lower BMIs reached at 12, 18 and 24 months of follow up. Furthermore, the percentage of IWL was 7higher in males ; this data appears considerably lower than reported in literature, probably due to the limited number of patients recruited and also due to the follow-up not superior of 2 years where pts are still within the so-called "*honeymoon phase from bariatric surgery*". This phase refers to a period following the procedure where patients typically experience significant weight loss and positive changes in their overall health, that usually occurs within the first 6 months to a year after the surgery. when weight loss is still strongly sustained by the surgical effect. (*Angrisani et. al 2015*) However male subjects are more subject to IWL and this is in line with what has been found in the literature (*El Ansari et al. 2021*). This feature can be explained by the higher incidence of Mets in men. T2DM in fact is associated with poor response in long-term follow-up. (*Foolad et al. 2022*) Another possible reason may be the influence of high cortisol level, found with higher incidence in males than females, in recent studies it has been showed that the percentage of total weight loss (TWL) after SG has a significant inverse correlation with serum cortisol level only in men (*Hironori et al. 2023*). Last but not least, we should also consider the influence excreted by steroid sex hormones in weight loss. In fact, as it is well known, severe obesity in males is often associated with low levels of testosterone and high serum estradiol levels due to the increased peripheral aromatization of androgens that results from increased body mass. (*Hammoud et al. 2010*)

In our series, we do not report any WR since as known in larger previously published series the WR after BS occurs generally after 2 years of follow up and can reach the 20% of pts after surgery.

As can be seen from our study of all the values taken into consideration, at 6 months of follow up only in females group, the levels of HbAc1 and BMI resulted correlated as a positive predictor of

EWL (p 0,03). However, always in females, the HbAc1 and BMI levels lose their significance (p 0,09 and 0,07) in favour of TG levels at longer follow up, as 24 months (P 0,003). This data can be interpreted considering that patients with Mets or ongoing glycaemic impaired control can benefit more from a BS during the immediate post-intervention interval due to the immediate metabolic effect on glycaemic control after surgery, while TG are normalized slower during the months after BS (*Sharma R. at al. 2016*) Only TG in women has a significant predictive value of long-term EWL, but not in men. We can suppose that women, who are less dysmetabolic, generally healthier and more affected by gynoid obesity than men, have a more responsive system to metabolic modifications. This hypothesis is in line with what was recently demonstrated by a Dutch study performed on a series of 3500 pts undergoing bariatric surgery, that showed that Men undergoing BS are more often affected by greater comorbidities that women and that all of these are in a more advanced state of the disease in men. (*Nienke van Olst et al. 2023*)

Looking at comorbidities resolution after BS and their influence on EWL between sexes, the condition of psychiatric syndrome and consequent psychiatric pharmacotherapy assumption before surgery have been shown to influence negatively the long-term EWL in both sexes (p 0,069 F; p 0,015 M). This is clearly explained by the possible low compliance to post-operative diet and the presence of alimentary disorders. For this reason pts should always be accurately evaluated by a multidisciplinary team before surgery in order to identify psychiatric disorders for a better selection of pts improving the postoperative outcome. (*Legatto et al. 2022*; *Sicob Guidelines 2023*)

Interestingly in our series, hypertension (considered as pts with anti-hypertensive therapy at the moment of BS) has a direct correlation with better EWL at 24 months. Without being able to exclude the fortuitous case, we could explain this result if we consider that patients that are already in a pharmacotherapy tend to be more compliant with behavioural changes and dietary rules after BS. However, we cannot also exclude the possibility of some influence linked to the biomolecular activity of antihypertensive drugs on EWL after BS.

As we showed in our series of bariatric pts no differences were found in EWL at 6 and 24 months between sexes when stratified by type of BS. This is in line with literature demonstrating the efficacy of SG, OAGB and RYGB that are the most diffused bariatric surgical operations to treat Obesity and obesity-related comorbidities. However it has been demonstrated that the correct choice of type of bariatric intervention in a contest of multidisciplinary team is fundamental for an optimal outcome. (*Sicob Guidelines 2023*)

Regarding GER as we know by evidence in literature SG is a high pressure gastric pouch system associated with up to 16% of Barret oesophagus diagnosis and GER during follow up (*Genco et al. 2017*), while OAGB has been associated to an incremented risk of biliary GER based on the type of intrinsic anatomical assembly of the intervention itself. In our series a worsening or "*de novo* " appearance of reflux symptoms after SG and OAGB were clearly highlighted, as well on the other hand also the complete remission or absence of new reflux symptoms for pts undergone RYGB (p= 0,006). In fact RYGB is considered an anti-reflux procedure in particular in obese pts. (*SAGES Guidelines 2021*)

Interestingly, in this study looking at the 61 OAGB pts, a complete remission of symptoms at 24 month was found for patients (n=4) with previous diagnosis of GER but in 13 patients they were highlighted "*deNovo*" GER symptoms with incrementations of pharmacological therapy. This effect could be linked to the fact that OAGB although it has a low pressure gastric pouch it has a longer and bigger gastric pouch and therefore rich in P cells potentially secreting acid. Another important aspect can be explained also by the intrinsic assembly of the OAGB which involves direct contact of the bile with the gastric pouch. (*Musella et al. 2021*)

In a smaller subgroup cohort of patients affected by severe obesity undergoing bariatric surgery, our study explored the AT production of APN as a potential predictive marker of EWL obtained with bariatric surgery. In females, we found that APN produced in abdominal VAT was positively associated with EWL starting from 12 months from surgery, while there was no significant correlation in operated males. Neither circulating nor abdominal SAT APN displayed any significant association with EWL in either sexes. In females, VAT APN levels in the upper quartile maintained a significant correlation with EWL at all the four time points of follow up considered, also at multivariate regression analysis adjusted for baseline BMI. Conversely, the ability of basal BMI to predict EWL was weaker, and lost its significance at multivariate analysis. W/H was associated with EWL only at longer follow up, losing any predictive power at short time, and when clustered for pear- and apple-shaped classes. A cut-off value for VAT APN in females able to predict the 80% EWL at 6 months and 95% EWL at 12 months was identified with a predictive threshold for identifying the best responders to bariatric surgery among female patients.

Our findings suggest that APN produced by VAT in women with severe obesity may represent an independent predictor of EWL. Obesity is characterized by lower circulating levels of this adipokine, which remains higher in females than in males. High circulating APN is correlated with a better insulin sensitivity and lower abdominal visceral adiposity. (*Lee et al. 2006)* The impact of sex and fat pad distribution on the AT local production of this hormone has been poorly investigated. This is particularly relevant in obesity, a condition characterized by a different sex-dependent distribution of the excess adiposity, which is generally concentrated in the visceral abdomen in males (apple-shaped) and in the subcutaneously peripheral fat (gluteal-femoral hip) in females (pear-shaped). (*Alser et al. 2022*) The subcutaneous abdominal and hip depots are metabolically healthier than the visceral depots (*Smith et al. 2017*), the latter characterized by hypertrophy associated with a reduced quality of the AT. (*Delaney et al. 2022*) These differences result in a different risk of obesity-associated comorbidities. (*Lee et al. 2006*) Notably, we found that while circulating APN was higher in females than in males with severe obesity, (*Lihn et al. 2004*) SAT and VAT levels were generally lower in females, with significantly higher APN in the male SAT. Interestingly, when we compared APN content in fat pads, according to pear- or apple-shaped distribution of AT in severe obesity, we found that the pear-shaped content of VAT APN is significantly higher compared to apple-shaped, in particular for the female sex. Taken together these findings suggest that VAT rather than SAT APN is the relevant marker of healthier fat even in severe obesity, in particular in females. In a previous study conducted in a nonhomogeneous cohort with only 40% of subjects with severe obesity, VAT mass was negatively correlated with APN secretion from *ex vivo* VAT explants (*Reneau et al. 2018*), or from *in vitro* cultured adipocytes isolated from VAT of women with normal weight. (*Drolet et al. 2008*) Similarly, to our data, these correlations were evident in females, confirming that VAT rather than SAT APN decrease reflects increased adiposity in a sex-related manner. Though the mechanisms responsible of this paradoxically inverse association, our findings suggest that in women, VAT expansion is associated with a relevant tissue disfunction, which may explain the greater detrimental effects of central adiposity and metabolic syndrome in women. (*Sarafidis et al. 2006*) Indeed, expansion of abdominal AT in females is characterized by different mechanisms according to the depots: while both hypertrophy and hyperplasia of adipocytes occur in SAT, fat deposition in visceral abdominal depots involves hypertrophic process resulting in altered AT functions and metabolism. (*Drolet et al. 2009*)

Circulating APN did not reflect abdominal AT production rather the contribution of subcutaneous gluteal adipose pads, which our data suggest to be deficient in men displaying apple-shape compared to women resulting in lower circulating APN.

Chapter 5

CONCLUSION

Obesity is a complex multifactorial disease and it needs to be treated in a multidisciplinary setting in order to better characterize the patients, in order to offer a more personalized therapy aslo at the level of surgery. Success after BS depends on many variables, and not all of them are yet investigated. Identifying sex-specific predictive factors to develop individualized treatment strategies for obese pts can have an important impact on clinical practice in order to obtain efficient weight loss for severe obesity.

Our data confirms as independent prognostic factors, serum TG and VAT adiponectin for EWL after BS but only in female subjects. In particular, our data demonstrate that EWL is associated with VAT and not with SAT APN content, with no correlation with APN serum levels. It can be hypothesized that the better quality of VAT may facilitate the reaching of the ideal weight after surgery. Of note, this relation is evident only in females. Moreover, females in the upper class of VAT APN performed better than males with the same levels of VAT APN in terms of weight loss during the first year from surgery. These findings further support the concept that APN is a penetrant marker of VAT quality, also contributing to weight normalization, but only in the female context, probably as depending on the sex hormone milieu. Accordingly, it could be of help measuring APN content in VAT samples obtained during surgery to improve the patient management, in particular a closer postoperative multidisciplinary follow-up protocol could be hypothesized for cases with reduced APN revealed by VAT biopsy at the time of surgery in order to improve the postoperative outcome.

Even more appealing could be creating a radiological identification of visceral fat with percutaneous biopsy to estimate the risk of a worse outcome for patient or maybe to identify the best surgical intervention.

We recognize some limitations of our study, which is retrospective and small, although it is homogenous, since bariatric surgery is performed monocentrically limiting any variance related to the type of surgery. The small power derived from the low number of participants may affect results, which need to be confirmed in larger prospective studies. Moreover, the confounding effect of the different pharmacological regimen of patients has not been taken into consideration; the follow-up was short (2 years) limiting considerations on APN predictivity on WR at long follow up, and reported only the endpoints of EWL and complications. Finally, the absence of collected glycemia, insulin, hypertensive, inflammation, and lipid profile data at follow up, prevents from any analysis of APN as a predictive marker of recovery from associated comorbidities.

In conclusion, our findings indicate that APN produced in the abdominal VAT rather than the circulating or subcutaneous levels can predict EWL after bariatric surgery as an independent factor only in the female sex. Similarly TG level revealed an independent factor that can predict EWL in female subjects. Thus leading to the concept of females that seems to be more adaptive to metabolic changes after bariatric surgery than males. If validated in larger cohorts of patients, these results may contribute to a better management of the patients with severe obesity, enabling to identify those patients who will benefit much more from surgery.

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