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METABOLIC REPROGRAMMING AFTER ROUX-EN-Y BARIATRIC SURGERY:

A ONE-YEAR FOLLOW-UP STUDY

Final Degree Project

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BIOCHEMISTRY AND MOLECULAR BIOLOGY | | BIOTECHNOLOGY

This project is based on the results obtained during my extracurricular internship with the Nutrition and Metabolism Research Group at the Dr. Peset University Hospital – Fisabio Foundation, under the supervision of Dr. Celia Bañuls Morant.

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1. ABSTRACT AND ABBREVIATIONS

ABSTRACT

Background: Roux-en-Y gastric bypass (RYGB) is an effective bariatric surgery for morbid obesity treatment that causes weight loss and induces body changes on a metabolic and molecular level. This research sought to assess the consequences of RYGB after a year in regard to clinical outcomes, paying closest attention to focus on the metabolic aspects, endocrine system, level of oxidative stress, and the bioenergetic processes occurring in the mitochondria.

Methods: Patients who had RYGB were included in the study population and were assessed both prior to and one year following surgery. First, we determined lipid, glycaemic, and anthropometric parameters and then, numerous gut-derived hormones (GIP, GLP-1, PP, PYY), adipokines (leptin, adiponectin, resistin, and adipsin), and PAI-1 using Luminex. We obtained oxidative stress parameters: advanced glycation end-products (AGEs), mitochondrial ROS (mtROS), and total reactive oxygen species (ROS) and DNA/RNA oxidative damage, using flow cytometry and Elisa's assays. Mitochondrial function in peripheral blood mononuclear cells was examined using flow cytometry to measure mitochondrial mass. The Cell Mito Stress Test Kit allowed us to determine oxygen consumption rate (OCR) measuring respiration and other mitochondrial parameters.

Results: Patients achieved significant weight loss, improvements in glycaemic, lipid profiles and reductions in systemic inflammation one year after the surgery. Leptin, GIP, PAI-1, and adipsin levels all decreased significantly and adiponectin levels increased. Total pool of ROS levels didn't change, but both mtROS and AGEs decreased significantly, suggesting lower oxidative damage. Also, DNA/ARN oxidative damage decreased. Furthermore, mitochondrial mass increased. We found an improvement in mitochondrial respiratory function, with increased basal respiration and maximal respiratory capacity.

Conclusions: RYGB surgery not only promotes substantial weight loss but also contributes to a metabolic and endocrine reprogramming. The remodeling of adipose tissue secretory patterns supports better appetite regulation, insulin sensitivity, and decreased systemic inflammation. Improvements in mitochondrial function and reduced mtROS and AGEs suggest attenuation of oxidative stress and potential protection against DNA damage. These findings reinforce the systemic benefits of RYGB and highlight its role in reversing key cellular mechanisms underlying obesity-related comorbidities.

ABBREVIATIONS

AGES – Advanced Glycation End Products

BMI – Body Mass Index

BS – Bariatric Surgery

DBP – Diastolic Blood Pressure

FCCP – Carbonyl cyanide-p-trifluoromethoxyphenylhydrazone

GIP – Gastric Inhibitory Polypeptide

GLP1 – Glucagon-Like Peptide-1

IFNγ – Interferon gamma

mtROS – Mitochondrial Reactive Oxygen Species

OCR – Oxygen Consumption Rate

PAI-1 – Plasminogen Activator Inhibitor-1

PBMCs- Peripheral blood mononuclear cells

PP – Pancreatic Polypeptide

PYY – Peptide YY

R/AA – Rotenone/ Antimycin A

ROS – Reactive Oxygen Species

RYGB – Roux-en-Y Gastric Bypass

SBP – Systolic Blood Pressure

SVF – Stromal Vascular Fraction

TNF-α – Tumor Necrosis Factor alpha

2. INTRODUCTION

2.1 OBESITY: DEFINITION, DIAGNOSIS, AND ASSOCIATED COMORBIDITIES

The World Health Organization (WHO) defines obesity as abnormal or excessive fat accumulation that presents a risk to health. To diagnose it, BMI (body mass index) is usually used, but this is not an accurate indicator of individual composition and metabolic risk (Piché et al., 2020). In order to be able to evaluate it more precisely, parameters such as visceral fat or the function of adipose tissue are being used. It's more important the distribution of the fat than the amount of fat. Patients with more abdominal fat present a negative metabolic profile (Lin & Li, 2021a). Comorbidities associated with obesity could be type 2 diabetes, hypertension, dyslipidemia, cardiovascular disease, osteoarthritis, chronic kidney disease, several types of cancer, and mental health disorders such as depression (Piché et al., 2020).

Obesity is global epidemic and its prevalence is increasing as it has tripled in the last 50 years. The World obesity atlas 2024 stated that 63% of people in the European region were overweight or obese in 2020. That number would rise to 66% by 2025 (Lobstein et al., 2024).

Internal and external factors contribute to obesity development and not only energy imbalance. These include social and environmental effects, as well as genetic, hormonal, and psychological aspects. Body weight variation is determined between 40% and 70% by genetics. Signaling pathways responsible for hunger and satiety, such as the leptin-melanocortin pathway, are altered in monogenic and polygenic variants of this disease (Lin & Li, 2021b). Other factors, including eating habits, microbiota, gastrointestinal hormones, and central nervous system activity have to be considered.

2.2 MOLECULAR MECHANISMS INVOLVED IN THE PATHOPHYSIOLOGY OF OBESITY

Molecular mechanisms involved in the pathophysiology of obesity have a more complex background than just a sedentary lifestyle or calorie excess. Complex and dysregulated pathways impact in oxidative balance, mitochondrial function, inflammatory responses, endocrine signaling, and genome integrity. The primary molecular axes that are crucial to the onset and progression of obesity will be examined in the following sections.

2.2.1 ENDOCRINE DYSFUNCTION OF ADIPOSE TISSUE IN OBESITY

The adipose tissue functions are energy reserve and endocrine regulation. It releases molecules that are responsible for energy metabolism and maintaining homeostasis. This endocrine organ releases adipokines responsible of responses that regulate inflammation, the immune system, the regulation of appetite and satiety, weight gain or insulin sensitivity. Hunger and satiety signals must be well

interpreted through the crosstalk that is established between adipose tissue, the hypothalamus and the digestive system (Hemat Jouy et al., 2024).

The endocrine function of adipose tissue is due to its composition. It is mainly formed by adipocytes responsible for the traditional function that was associated with adipose tissue, that is the traditional function of energy reserve. The metabolic functions are due to the stromal vascular fraction (SVF) and it is composed of immune system cells (macrophages, lymphocytes, mast cells), endothelial cells and preadipocytes. Subcutaneous and visceral fat can be distinguished depending to their function. The first is metabolically active and has energy reserve, thermal insulation and protective functions. It synthesizes hormones such as leptin and adiponectin. The second is metabolically low and is related to a higher cardiometabolic risk (An et al., 2023).

In greater lean adipose tissue individuals predominate immune cells with regulatory and immunosuppressive functions. When the mass of adipose tissue increases due to an imbalance in nutrient intake and expenditure, the profile changes. Obesity alters the behaviour of adipose tissue, realizing the amount of adipokines, and this change causes a metabolic alteration. A release pattern of pro-inflammatory, atherogenic and diabetogenic factors is developed (Taylor, 2021).

Inflammatory adipokines (leptin, resistin, lycopocalin and adipsin) increase while anti-inflammatory ones (adiponectin) are decreased in obesity. Other hormones such as insulin, ghrelin, GIP (Glucose-dependent Insulinotropic Polypeptide), GLP-1 (Glucagon-Like Peptide-1), PP (Pancreatic Polypeptide) and PYY (Peptide YY) are also altered. These six have enteropancreatic origin and are not secreted by adipose tissue. However, they can functionally interact with adipose metabolism, for example by regulating appetite, lipogenesis or insulin sensitivity (Hemat Jouy et al., 2024). See *Supplementary Material Table 1* for more information and adipokines functions.

This alteration does not only involve adipokines but molecules from other systems such as the renin-angiotensin-aldosterone system, involved in the increase in blood pressure, the fibrinolytic system or the complement system. Regarding the fibrinolytic system, PAI-1 is secreted by visceral adipose tissue. It participates in fibrinolysis and in the differentiation of adipocytes (*Figure 1*). Its levels are stimulated by pro-inflammatory molecules (TNF-α, TFG-β and free fatty acids) in obesity. The complement system is a complex system of enzymatic cascades activated by different pathways. Obesity and insulin resistance cause an increase in circulating levels of C3 and C4 of the classical pathway. Ingested and circulating lipids would increase the levels and the risk of coronary insufficiency. In obesity, the levels of adipsin and factor B, an inflammatory factor of the alternative complement pathway, are also increased (Zorena et al., 2020).

The changes that occur in adipose tissue configure an altered secretory profile. Chronic inflammation and metabolic dysfunction are promoted. A stage is set for chronic low-grade inflammation and oxidative stress. They will play a key role in the development of chronic diseases and metabolic complications.

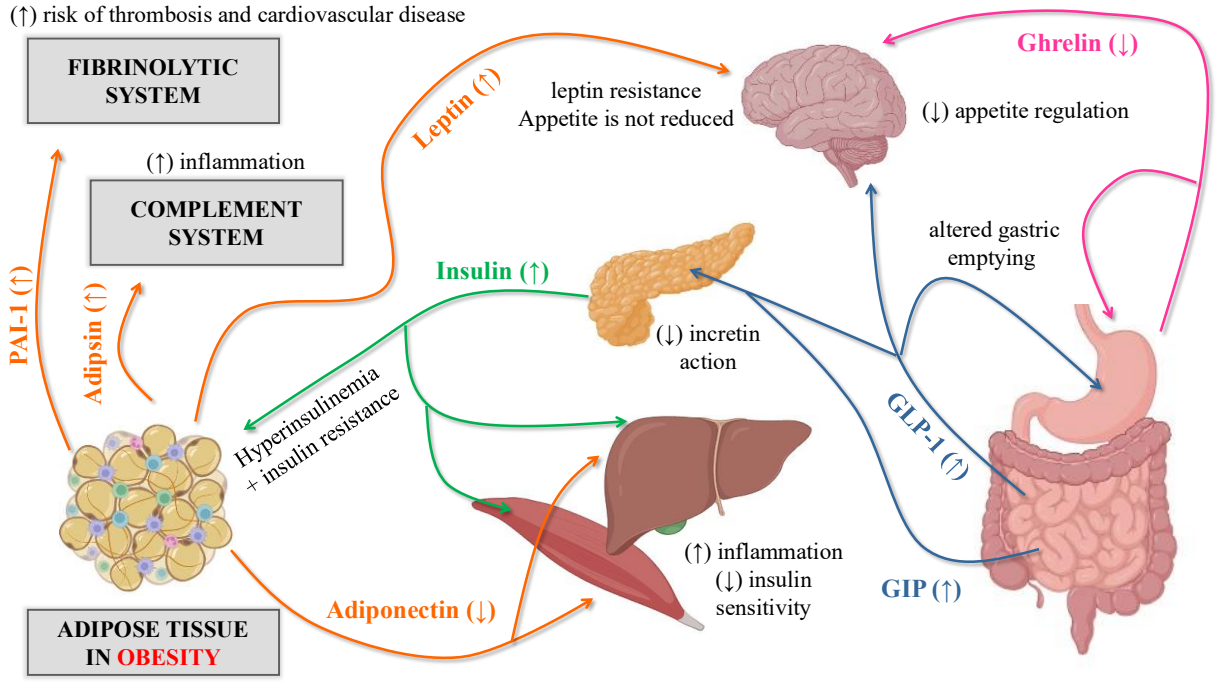

Figure 1. Alteration of the endocrine function of adipose tissue in obesity. *The secretory pattern of adipokines from adipose tissue is represented (orange arrows), which alters appetite regulation, leads to resistance to various hormones—preventing them from exerting their functions—and affects other systems, contributing, among other responses, to increased inflammation. The crosstalk with other organs is also shown: hyperinsulinemia and reduced insulin sensitivity (green); altered incretin function by GLP-1 and GIP (blue); ghrelin release and impaired appetite regulation (pink).*

2.2.2 Low Grade Inflammation and Oxidative Stress

Obesity presents chronic low-grade inflammation and oxidative stress. These two amplify each other and participate in the development of the disease. Excess intake can lead to fat accumulation. Adipose tissue grows, causing its enlargement. These changes are the size of adipocytes (hypertrophy) and the number of adipocytes (hyperplasia).

This growth exceeds the vascular capacity of the tissue, and, in the presence of poorly oxygenated areas, hypoxia occurs. Adipose tissue releases pro-inflammatory adipokines such as leptin and resistin in response in order to recruit immune cells. Macrophages release pro-inflammatory cytokines and ROS. The most abundant are IL-6 and TNF-α. Also, they inhibit anti-inflammatory adipokines (adiponectin or IL-10) that regulate the immune response and insulin sensitivity (Taylor, 2021).

The macrophage population increases and polarizes. In healthy adipose tissue, M2 predominates (responsible for the proliferation and differentiation of adipocytes generating a tissue that preserves insulin sensitivity). In the pro-inflammatory niche, M1 macrophages predominate, which maintain inflammation and lead to adipocytes to become resistant to insulin's antilipolytic effect. Lipolysis increases and free fatty acids are released into the circulation. They accumulate in non-fat tissues, causing lipotoxicity and tissue damage (Mancini et al., 2016).

There is an increase in ROS by the adipose tissue with this pro-inflammatory response. At the same time, excessive nutrient intake causes mitochondrial dysfunction. Oxidation is stimulated and mitochondrial ROS increase. Other sources of ROS are oxidases, protein kinase C, nitric oxide synthase (NOS), advanced glycation end-products (AGEs) and autophagy dysregulation. Oxidative stress involves an overproduction of ROS and a decrease in antioxidant defence (Mancini et al., 2016).

Inflammation increases ROS levels but, ROS can activate inflammatory pathways. A vicious cycle of amplification is established that promotes this chronic state and alters metabolism (*Figure 2*).

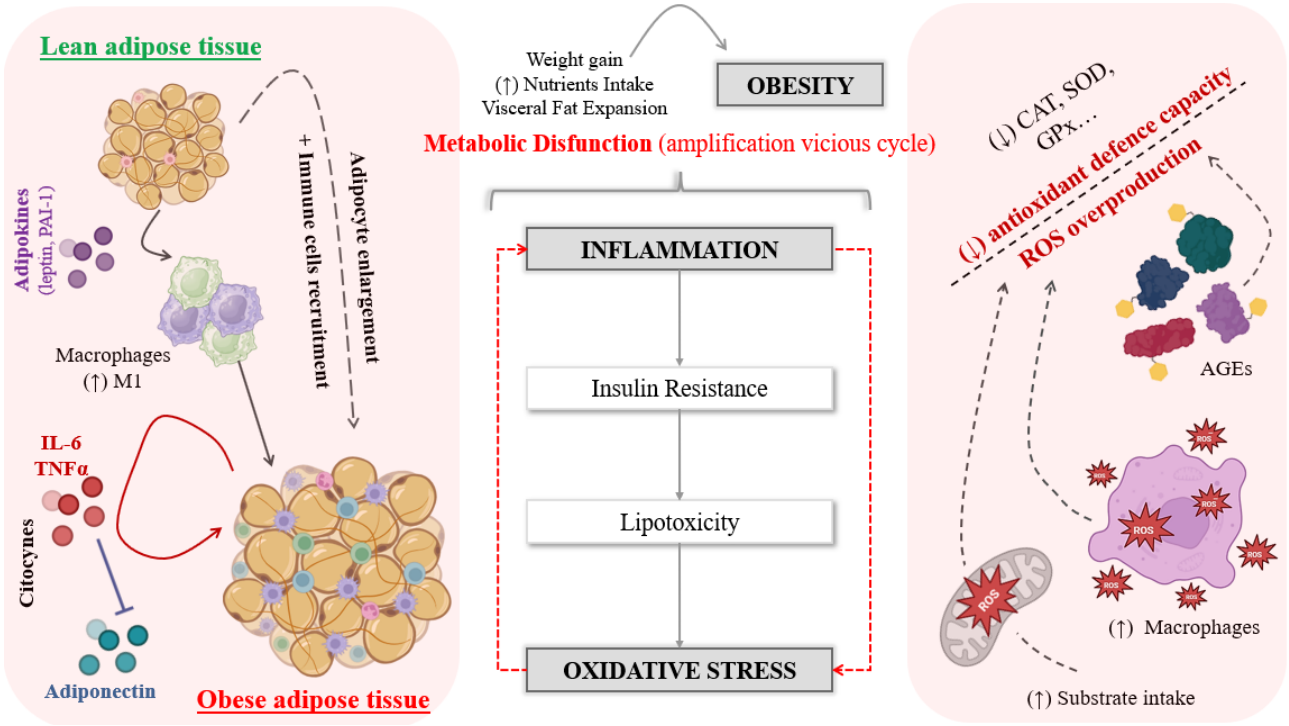

Figure 2. Feedback loop between inflammation and oxidative stress in obesity. *Endocrine dysfunction of adipose tissue leads to the release of pro-inflammatory adipokines, which recruit immune cells that secrete pro-inflammatory cytokines and inhibit anti-inflammatory ones. This increase in inflammation contributes to insulin resistance and lipotoxicity, which further elevate oxidative stress. The reduction in antioxidant defences and the overproduction of reactive oxygen species (ROS) from various sources result in increased systemic inflammation. An amplification loop is thus established between low-grade inflammation and oxidative stress driven by obesity.*

2.2.3 DNA/RNA Oxidative Damage

Both conditions often linked to obesity, the overproduction of reactive oxygen species (ROS) and the state of low-grade chronic inflammation have been shown to compromise genomic integrity. Among the primary causes of oxidative DNA damage—affecting both nuclear and mitochondrial DNA—and involved in cellular ageing as well as the onset of chronic diseases including cancer, these variables are taken into account (Włodarczyk & Nowicka, 2019).

DNA nitrogenous bases can be directly oxidized as a result of mitochondrial respiration and inflammatory responses. This results from the overproduction of ROS causing single- and double-strand breaks and thereby accelerating mutations and genomic instability. Such damage can interfere with DNA replication and transcription, therefore changing conventional cellular function (Chiaramonte et al., 2023). Furthermore, the progressive deterioration of mitochondrial DNA (mtDNA) is especially pertinent since mitochondria lack nucleotide excision repair processes, therefore increasing the vulnerability of mtDNA (Usman & Volpi, 2018).

DNA damage results from more than only oxidative stress. Obesity-related inflammation is crucial in this progress. Activated macrophages and other immune cells release cytokines like TNF- α and IL-6, which can cause DNA damage and promote carcinogenesis. Similarly linked to a raised cancer risk in numerous organs are low levels of adiponectin, an anti-inflammatory adipokine secreted by adipose tissue.

People with metabolically unhealthy obesity have demonstrated higher markers of oxidative stress—specifically total oxidation state and lipid hydroperoxides—as well as shorter telomeres than those with a metabolically good profile (Lejawa et al., 2021). These findings support the theory that oxidative stress in obesity might accelerate genomic degradation and cellular aging.

Among the most typical oxidative lesions are 8-hydroxyguanine (8-OHdG), 7,8-dihydro-8-oxoguanine, thymine glycol, Fapy-Ade, and Fapy-Gua. Among them, 8-hydroxy-2'-deoxyguanosine (8-OHdG) is one of the most widely used biomarkers for assessing oxidative DNA damage. Although several research have tried to link body mass index (BMI) and 8-OHdG levels, the findings are still contradictory. While some studies show a favourable relationship between obesity and increased 8-OHdG levels, others find no connection or even notice greater levels in lean people. The existence or absence of metabolic syndrome (MS) or insulin resistance—both of which can affect oxidative stress and the body's antioxidant capacity (Chiaramonte et al., 2023)—may explain these differences. Other in vitro studies suggest that hyperglycaemia itself can lead to DNA damage and mutations, and AGEs can promote DNA strand breaks and 8-OHdG accumulation. Even so, the presence of 8-OHdG in

biological samples like urine, serum, or saliva makes it a valuable and non-invasive biomarker for the early detection of carcinogenic processes and accelerated ageing, particularly in populations with obesity (Lejawa et al., 2021).

2.2.4 MITOCHONDRIAL BIOENERGETIC PROFILE AND DISFUNCTION

The main function of mitochondria is to provide energy to cells in the form of ATP. They play a key role in cell bioenergetics and in other functions such as apoptosis, calcium regulation, cell signaling, and the response to oxidative stress. Their structural and functional integrity maintenance is critical for overall cell health (Johannsen & Ravussin, 2009).

Pathophysiological conditions in obesity are linked to mitochondrial dysfunction. It is characterized by a reduction in ATP production, an increase in ROS, the modifications of fusion and fission dynamics of the mitochondria, the reduction in mitochondrial biogenesis, and inefficient mitophagy (Tang et al., 2024). Moreover, the obesity disease has been found to reduce mitochondrial oxidative activity, particularly in skeletal muscle and adipose tissue. Also, a lower expression of key enzymes in β-oxidation and the electron transport chain. This significantly compromises mitochondrial respiration and the capacity to generate energy.

The imbalance between intake and energy expenditure, causes an accumulation of nutrients that will be substrates for the oxidative phosphorylation chain. This chain saturates and perturbation of mitochondrial metabolism gives rise to ROS overproduction. Molecules such as hydrogen peroxide are generated that oxidize the cellular environment. This partial contribution to total ROS pool destroys insulin signaling and increases inflammation (Prasun, 2020).

As for mitochondrial mass, in obesity, the number and quality of functional mitochondria are reduced. It could be attributed to lower biogenesis (PGC-1α, SIRT1 regulators) and defective mitophagy – damaged mitochondria are not efficiently eliminated; hence their accumulation and overall mitochondrial efficiency decreases as a result (Tang et al., 2024). This in turn leads to a vicious cycle of energetic deterioration plus inflammation plus systemic metabolic dysregulation.

This mitochondrial respiratory dysfunction is not only a consequence of obesity but can also precede and exacerbate metabolic disorders. One would be insulin resistance, by reducing the oxidative phosphorylation efficiency and promoting intracellular lipid accumulation (de Mello et al., 2018). To better understand the role of mitochondria in metabolic disease, it may be essential to study mitochondrial respiration and other indicators of mitochondrial function, such as mitochondrial mass, ATP production, and rate of oxygen consumption.

2.3 THERAPEUTIC APPROACHES TO OBESITY

Obesity is a chronic, progressive, and a complex disorder; appropriate management should combine various treatment modalities. The program should be tailored according to persons objectives and health issues. Lifestyle changes like an adequate diet, more exercise, tackling habits and behaviour should be the first step. These adjustments can lead to a 10-percent loss of total body weight, which already has health benefits (Lejawa et al., 2021). But this is often not sufficient to maintain the weight in the long run, as the body tends to return to its original weight (Perdomo et al., 2023a).

Other treatments, such as drugs or surgery, may be necessary as part of a combined plan. Lifestyle changes that don't go quite far enough are supplemented by the second line of approach: the potentiating force of pharmacological treatment, especially in those with a BMI of 30 or more, or 27 if they have other health problems related to weight, like diabetes or heart disease.

These drugs generally act in the brain to decrease appetite or induce feelings of fullness. For example, orlistat prevents fat absorption, and phentermine increases certain brain chemicals to curb appetite (Lin & Li, 2021b). There are also new drugs being developed that act in far more sophisticated ways. They dictate how the body metabolizes glucose, burns fat or expends energy.

When lifestyle change and medications do not achieve the necessary results, bariatric surgery is an alternative, particularly for those with a BMI of above 35, or between 30 and 35 who have major issues like out-of-control diabetes. Surgery may lead to weight loss and also benefit other conditions such as sleep apnoea or fatty liver (Perdomo et al., 2023a).

The most frequent options include sleeve gastrectomy and Roux-en-Y gastric bypass (RYGB). In the RYGB procedure, the stomach is reduced in size, and a portion of the small intestine is bypassed, shortening the path of food. This restricts both the amount of food ingested and the absorption of nutrients. This surgery stands out for its long-term results (M.-E. Piché et al., 2020). Surprisingly, within days of the intervention, many patients with diabetes experience improvements in their glucose levels, even before significant weight loss. The reason for this is that the surgery affects the hormones in the gut, and right after the operation, people are eating very little.

The Roux-en-Y gastric bypass (RYGB) procedure alters the way body manage adipose tissue. These changes in adipose tissue are due to a cascade of physiological adaptations. This multifaceted process is characterized by a reduction in the dimensions of subcutaneous adipocytes. Then, the secretion of beneficial immunomodulatory factors increased. Adiponectin and various anti-inflammatory cytokines resume their functions. Consequentially, peripheral insulin sensitivity is potentiated, and homeostatic regulation of blood glucose concentrations is generally improved (*Figure 3*).

Furthermore, empirical investigations have consistently demonstrated that RYGB confers a more durable reduction in body mass compared to sleeve gastrectomy. This surgical intervention is additionally correlated with statistically significant improvements in subjective assessments of life quality, as well as beneficial modulations in circulating lipid profiles (Perdomo et al., 2023b). It remains critically important to acknowledge the potential for adverse sequelae, particularly in the context of sleeve gastrectomy. In select instances subsequent to RYGB, a secondary surgical intervention may be clinically indicated to mitigate complications such as gastroesophageal reflux, cholelithiasis (gallstone formation), or specific micronutrient deficiencies. Cases of weight gain or persistence of metabolic problems can also be observed (Carmona-Maurici et al., 2020). When other alternatives for addressing obesity have been unsuccessful, bariatric surgery, especially RYGB, remains an effective and safe therapeutic option.

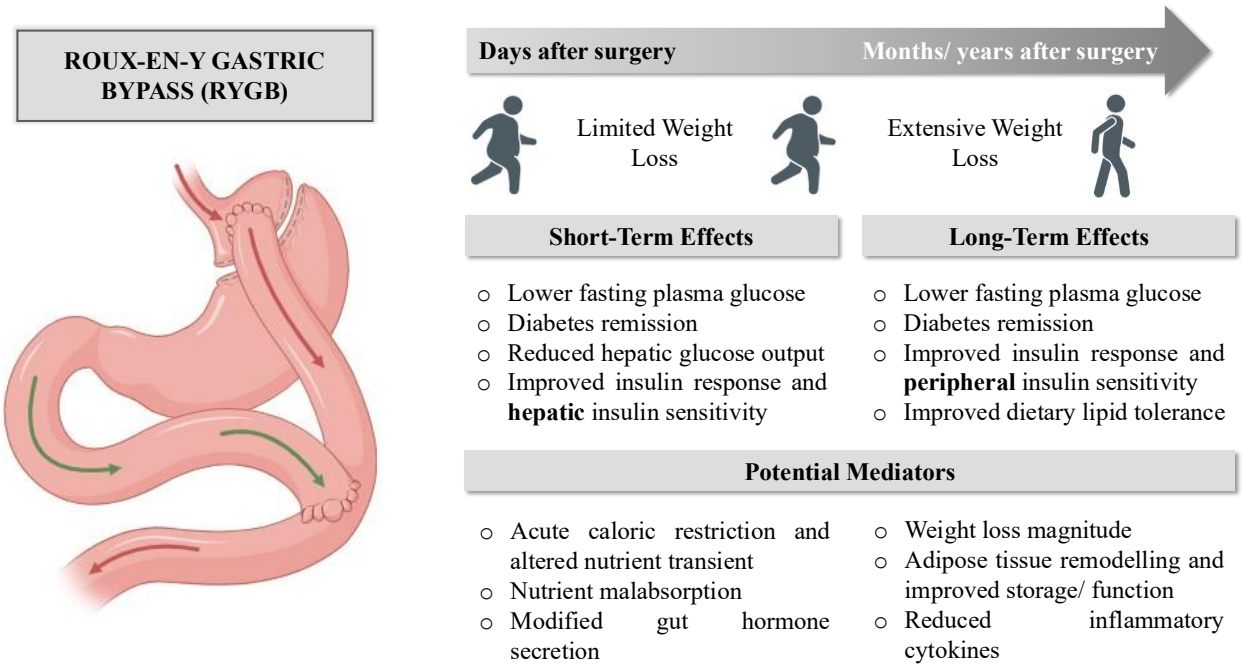

Figure 3. *Roux-En-Y Gastric Bypass (RYGB) representation. Short and long-term effects and the potential mediators due to the surgery intervention are shown in the figure. Adapted from M. E. Piché et al., 2020.*

3. HYPOTHESIS AND OBJECTIVES

This Final Degree Project goes beyond considering obesity treatment as simply weight loss, as it focuses on studying the cellular changes involved in reversing obesity. In fact, it is crucial to determine whether this surgical intervention, carried out in individuals with a high BMI and difficulties following better effective habits, could modulate altered metabolic pathways and protect against comorbidities.

Based on these premises, the hypothesis of this work is that bariatric surgery (RYGB) induces significant weight loss, leading to improvements in traditional clinical parameters (blood pressure, glycaemic and lipid profile), and, additionally, it may modulate key molecular mechanisms involved in the pathophysiology of obesity.

To confirm this hypothesis, the following specific objectives were proposed:

1. To evaluate the clinical effectiveness of bariatric surgery in weight loss and its impact on anthropometric, metabolic clinical parameters after 12 months of follow-up.
2. To determine the effect of the RYGB on the endocrine function alteration induced by obesity.
3. To study the impact of the surgical intervention on inflammation and oxidative stress status.
4. To assess one-year postoperative changes in mitochondrial function and bioenergetic profile of peripheral blood monocyte cells.

4. MATERIALS AND METHODS

4.1 EXPERIMENTAL DESIGN

For the completion of the following Final Degree Project, an interventional, clinic-basic, comparative, and prospective study was conducted. To achieve the proposed objectives, patients with obesity who underwent bariatric surgery at the General Surgery and Digestive System Service of the Doctor Peset University Hospital in Valencia were selected. All of them were informed regarding the objective and methodology, signing the informed consent. Personal data and the obtained results were anonymous processed and were managed in accordance with the Organic Law on Data Protection (LOPD). The study protocol was approved by the Human Ethics Committee of the Hospital (code 96/16), in accordance with the guidelines of the Declaration of Helsinki.

Patients were included in the study based on the following criteria:

- Inclusion criteria: Patients with a BMI greater than 40 kg/m² (or 35 kg/m² with at least one comorbidity associated to obesity), aged between 18 and 65 years, and a known disease progression of more than five years (and previous failure with dietary intervention and pharmacological treatment).
- Exclusion criteria: Due to the nature of the study, all patients with acute or chronic inflammatory diseases, liver and kidney failure, neoplastic diseases, and secondary causes of obesity (hypothyroidism, Cushing's syndrome) were excluded.

The parameters measured for the study were obtained before the intervention (baseline or pre-surgery) and after one year following Roux-en-Y Gastric bypass (RYGB) intervention (12 months or post-surgery).

4.2 METHODOLOGY

The following sections detail the methodology used to obtain patient samples before and after RYGB, as illustrated in *Figure 4.*

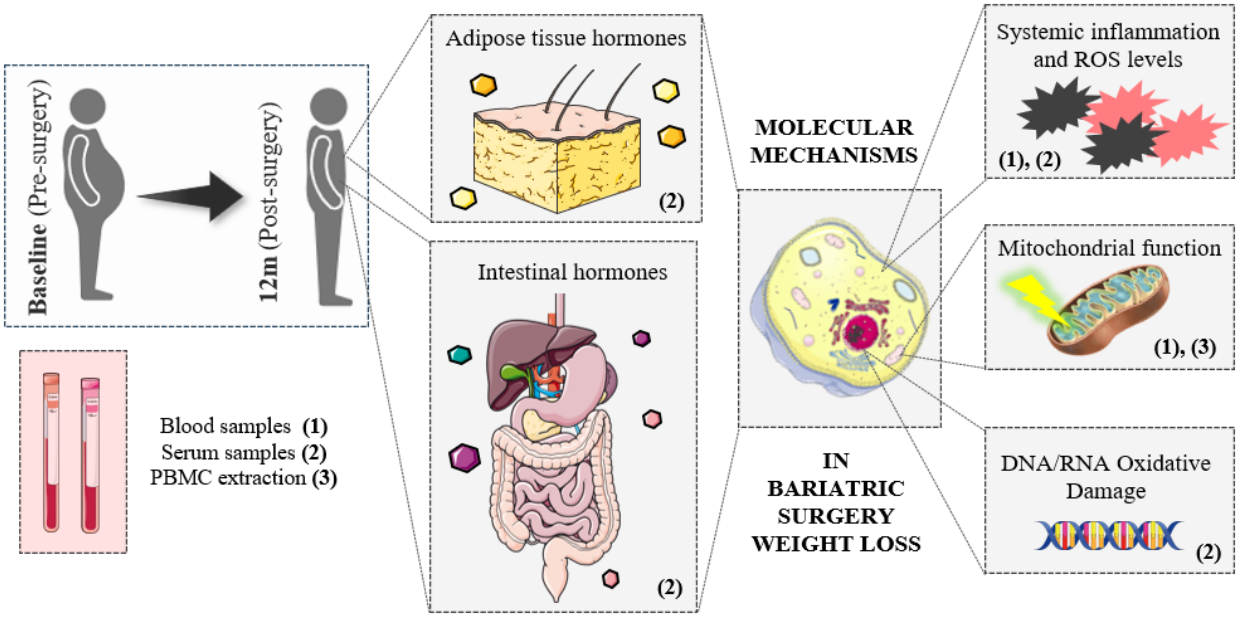

Figure 4. Graphical abstract of the methodology followed. *Sample collection and parameter measurement were conducted before and 12 months after bariatric surgery (RYGB). Additionally, the molecular determinations performed, and the type of sample used in each case are detailed.*

4.2.1 SAMPLE COLLECTION AND LABORATORY TESTS

Anthropometrics measurements were obtained during pre- and post-surgery physical explorations. Weight, height, systolic and diastolic blood pressure (SBP and DBP) and waist circumference were obtained by means of electronic scales, stadiometer, sphygmomanometer, and measuring tape, respectively. In case of waist circumference, it was measured in the midpoint between the lower border of the rib cage and the iliac crest in a standing position. BMI and the percentage of weight loss were calculated using *Formula 1* and *Formula 2.*

Formula 1. $$\boldsymbol{BMI = \frac{weight}{height^2}}$$

Formula 2 $$\boldsymbol{PWL = \frac{preoperative\ weight - current\ weight}{preoperative\ weight} \cdot 100}$$

Body composition (% of fat mass and lean mass) was determined by bioelectrical impedance analysis assessment, using an eight-electrode multifrequency impedance analyser (seca® mBCA 515/514).

Blood samples were collected from the antecubital vein in fasting conditions at 8:00-9:00 a.m. at baseline and one year after RYGB surgery. One part of the samples was destinated to the Hospital's Clinical Analysis Service where the biochemistry determinations were performed.

In order to evaluate the glycaemic markers, glucose was determined by enzymatic assay; insulin was determined by an immunochemluminescence assay (Abbott, Chicago, IL, USA) and the Homeostatic Model Assessment for Insulin Resistance Index (HOMA-IR) was calculated with the *Formula 3*. The percentage of glycated haemoglobin (HbA1c) was obtained with a glycohemoglobin analyser (Arkray Inc., Kyoto, Japan). In case of evaluating the lipidic markers, total cholesterol (TC), and triglycerides (TG) serum levels were determined by enzymatic assay; HDL cholesterol (HDLc) concentration was measured using Beckman LX20 analyser (Beckman Coulter Inc., Brea, CA, USA); LDL cholesterol (LDLc) was calculated by Friedewald's formula when circulating levels of TG did not exceed 300mg/dL; and the apolipoproteins Apo-A1 and Apo-B with nephelometry. The atherogenic index of plasma (AIP) was calculated using the *Formula 4* .

The emergent markers were also evaluated. Systemic levels of high sensitivity C-reactive (hsCRP) and C3 fraction of the complement (C3c) were analysed using an immunonephelometric assay (Behring Nephelometer II, Dade Behring, Inc.; Newark, DE, USA) with an intra-assay coefficient variation <5,5 %; homocysteine (chemiluminescent microparticle immunoassay on the Alinity i analyzer by Abbott) and fibrinogen (immunologic/chromogenic method on the STA R Max 3 analyzer by Stago).

Formula 3 $$HOMA - IR = \frac{fasting\ insulin\ (\mu UI/mL) \cdot fasting\ glucosa\ (mg/dL)}{405}$$

Formula 4. $$AIP = log\frac{TG}{HDL}$$

The other portion of the samples was taken to the hospital laboratory for further experimental determinations. Serum was obtained by centrifuging the blood sample tubes without anticoagulant at 1500 g and 4ºC for 10 minutes. In cases where serum with inhibitors was needed, Pefabloc (Sigma) 100 mM and DPPIV (Sigma) 50 μM were added to the blood samples and incubated at room temperature for 20 minutes before being centrifuged. The serum aliquots, both with and without inhibitors, were immediately stored at -80 °C for immunoassay analyses.

4.2.2 IMMUNOASSAYS

Three different immunoassays were conducted to analyse human cytokines, Advanced Glycosilated End Products (AGEs) and oxidative damage in DNA/RNA.

First, the **Luminex® 200 analyser system** (Luminex Corporation, Austin, TX, USA) was used to quantify serum levels of pro-inflammatory molecules (IFNγ, IL-10, IL-17, IL-1B, IL-2, IL-4, IL-23, IL-6, IL-8 and TNF-α). Then, other cytokines were analysed with a Luminex kit (ghrelin, GIP, GLP1, leptin, PP and PYY) and with another kit (adiponectin, lipocalin, resistin, adipsin and PAI-1). In all cases, the procedures of the Milliplex® kit manufacturer were followed; MILLIPLEX ® Human High Sensitivity T Cell Magnetic Bead panel, The MILLIPLEX ® Human Metabolic Hormone Magnetic Bead Panel and MILLIPLEX® Human Adipokine Magnetic Bead Panel respectively.

Theses assays allow analyse multiple biomarkers, such as cytokines, from a single serum sample (in each protocol was indicated if serum inhibitor was required). It uses **MagPlex®-C microspheres**, which are magnetic beads that are color-coded with fluorescent dyes.

Each bead set is uniquely coloured using two fluorescent dyes, which enables the detection of multiple analytes at once. The beads are coated with specific antibodies that capture the target molecules, such as cytokines, present in the serum sample. Once the analyte is captured by the bead, a biotinylated detection antibody is introduced to bind to the captured analyte. The reaction is then completed with a Streptavidin-PE conjugate, which acts as a reporter molecule.

After incubation, the beads are analysed using Luminex's proprietary system that measures the fluorescence emitted from each bead. The unique colour of each bead corresponds to a specific analyte, which allows the system to identify and quantify multiple cytokines in the same sample.

Second, an **ELISA assay** was conducted to measure AGEs, the commercial Human Advanced Glycation End Products (AGEs) ELISA Kit from Abebio was used. This assay employs a two-site sandwich ELISA to quantify AGEs, using a pre-coated microplate to immobilize the analytes.

First, 100 µL of sample or standard were added to each well and incubated for 2 h at 37 °C. After incubation, the wells were washed with 250 µL of Wash Buffer to remove unbound substances. Then, 100 µL of biotin-conjugated antibody specific for AGEs was added and incubated for 1 h at 37 °C. Following another wash step, 100 µL of streptavidin-conjugated horseradish peroxidase (HRP) was added. Unbound enzyme conjugates were removed by washing. Then, 100 µL of Substrate Solution was added to each well, and colour development occurred in proportion to the amount of AGEs bound in the initial step. The plate was incubated for 15–20 min, and the reaction was stopped by adding 50 µL of Stop Solution. The intensity of the colour was measured at 450 nm using a microplate reader.

Third, an **ELISA assay** was conducted to evaluate the DNA/RNA oxidative damage quantifying three types of oxidized guanines. In this case, the "Cayman Chemical ELISA kit" procedure was followed.

Serum samples without inhibitor were diluted 1:25 with Elisa Buffer 1X. To perform the assay in a 96 well-plate, it was necessary to add the reagents depending on the well type: standard curve, blank (Blk), NSB (Non-specific binding), TA (Total Activity) or sample. The plate was incubated overnight (4ºC). Then, wells were rinsed five times with Wash Buffer. Reconstituted Ellman's Reagent was added to each well, and tracer to TA wells. Using an orbital shaker for 90 min, the previously covered plate was incubated. Finally, absorbance was read in a wavelength between 405-420 nm.

4.2.3 FLOW CYTOMETRY

Oxidative stress markers were assessed using a flow cytometry assay (Accuri C6, BD Biosciences, Franklin Lakes, NJ, USA). Whole blood samples (500 µL) were treated with Red Blood Cell Lysis Solution (Miltenyi Biotech, Bergisch Gladbach, Germany) to remove erythrocytes, followed by centrifugation. The remaining cell pellet was resuspended in 200 µL of HBSS (Hank's Balanced Salt Solution) and incubated with 4 µL of allophycocyanin (APC) anti-human CD45 antibody (Invitrogen, Life Technologies, OR, USA) for 20 min in the dark to label leukocytes.

After labeling, samples were diluted (1:10) and incubated for 10 min with the appropriate fluorescent probes for specific oxidative stress measurements. Total reactive oxygen species (ROS) and mitochondrial ROS were detected using 2′,7′-dichlorodihydrofluorescein diacetate (5 µM, Invitrogen, Life Technologies, OR, USA) and MitoSOX Red (3 µM, Invitrogen, Life Technologies, OR, USA), respectively. Lastly, for mitochondrial mass, Mitotracker dye (0,3 µM, Invitrogen™ MitoTracker™ Green FM Dye) was used.

4.2.4 PBMCs ISOLATION FROM BLOOD SAMPLES

Blood collected in BD Vacutainer ® citrated tubes were processed according to MACSprep™ PBMC Isolation Kit, human (Ref. 130-115-169, Miltenyi) procedure, by two separation steps at room temperature (RT). First, erythrocytes are aggregated and sedimented and then, PBMCs are isolated by depletion of non-PBMCs (neutrophils, eosinophils, platelets, and residual erythrocytes). A cocktail of biotin-conjugated monoclonal antibodies and MACSprep™ Anti-Biotin MicroBeads are used for indirectly magnetically labeling the non-PBMCs and separating them with a magnetic field in a column. The resulting pellet was washed and resuspended with Seahorse medium.

4.2.5 REAL-TIME METABOLIC FLUX ANALYSIS

Determination of mitochondrial functions of pre- and post-surgery PBMCs was assessed in real-time with a Seahorse XFp analyzer (Agilent) using the XFp Cell Mito Stress Test Kit (Agilent).

Extracted PBMCs were resuspended with Seahorse XF DMEM (Dulbecco's Modified Eagle Medium) medium pH 7.4 (Agilent) supplemented with glutamine 2mM, 10 mM glucose and 1mM

pyruvate. Day prior to assay, the sensor cartridge was hydrated with Poly-D-Lysine (0.1 mg/ml) and miliQ water and incubated overnight at 37 ºC, no CO_2.

The day of XF Assay, PBMCs were seeded at a density of $3x10^5$cells per well. Assay reagents were loaded in the cartridge ports following the manufacturer's volume recommendations (Oligomycin A 1.5 µM, FCCP 1.0 µM and Rotenone/Antimycin 1 µM) see *Figure 5*. Results were obtained and processed by the Analyze XF Assay Results and Seahorse Analytics software application, and data was normalized from 300000 to 250000 cells.

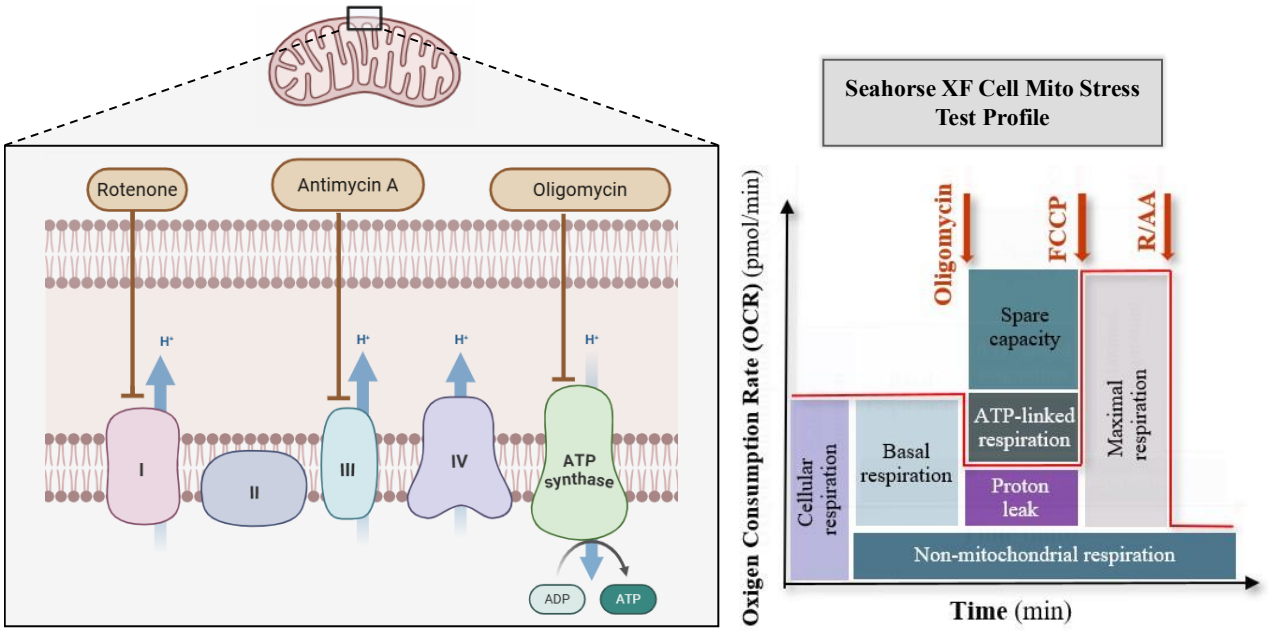

Figure 5. Real-time metabolic flux analysis: assay principles. *The assay for determining various mitochondrial parameters as oxygen consumption rate (OCR) (pmol/min) units, involves the sequential injection of inhibitors into PBMCs from obese patients at different time points.* ***1st injection****: oligomycin inhibits ATP synthase (complex V).* ***2nd injection****: carbonyl cyanide-4 (trifluoromethoxy) phenylhydrazone (FCCP) collapses the proton gradient and disrupts mitochondrial membrane potential.* ***3rd injection****: a mixture of rotenone, which inhibits complex I, and antimycin A, which inhibits complex II.*

4.2.6 Statistical Analysis

For data analysis, the statistical software SPSS 22.0 (IBM SPSS Statistics, Chicago, IL, USA) was used. To determine whether the data followed a normal distribution, a normality test was conducted to distinguish between parametric and non-parametric parameters. Since this study included a sample size of 25 patients, the Shapiro-Wilk test was used. When the normality test yielded a p-value < 0.05 (indicating a non-parametric parameter), the Wilcoxon test (for two paired samples) was performed. Otherwise, the statistical test used was the T-test (for two paired samples). For parameters with

normally distributed data, results are expressed as Mean ± Standard Deviation. In contrast, for those without a normal distribution, data is presented as Median (25th percentile – 75th percentile).

To correlate the percentage of weight loss with the other obtained variables, a Pearson or Spearman correlation was conducted for parametric or non-parametric parameters respectively.

The data was graphical represented using GraphPad Prism and Figures were made using BioRender and Medical Art Pictures.

5. RESULTS

5.1 WEIGHT LOSS IN TERMS OF ANTHROPOMETRIC AND BIOCHEMICAL PARAMETERS

After the recruitment period, a cohort of 25 patients who underwent bariatric surgery was obtained (*Table 1)*. These individuals had a mean age of 46.79 ± 11.28 years, with approximately 80% being women. Regarding body weight before the intervention, the mean value was 103.24 ± 12.94 kg, which significantly decreased to 73.08 ± 9.58 kg one year after surgery. To quantify weight loss, the percentage of weight loss (PWL%) was calculated, resulting in 29 ± 6.04%.

In parallel with the weight loss induced by RYGB, physical examinations revealed a significant reduction in waist circumference, body fat percentage, and visceral fat, while lean mass percentage increased. The effect of weight loss on blood pressure was also evaluated, showing a significant decrease in both SBP and DBP values compared to baseline ones.

Table 1. Anthropometric parameters in obese patients (n=25) before and after the intervention based on bariatric surgery.

ANTHROPOMETRIC PARAMETERS			
	PRE-SURGERY (Baseline)	**POST-SURGERY (12m)**	**p-value**
n	25	-	-
Women % (n)	80% (20)	-	-
Age (years)	46.79 ± 11.28	-	-
Weight (kg)	103.24 ± 12.94	73.08 ± 9.58	<0.001
BMI (kg/m^2)	37.80 ± 3.90	26.50±6.80	<0.001
Waist (cm)	98.70 ± 8.00	79.60 ± 10.30	<0.001
Fat mass (%)	47.20 ± 4.90	33.20 ± 8.20	<0.001
Lean mass (%)	52.80 ± 4.90	66.80 ± 8.20	<0.001
Visceral fat (L)	3.23 ± 0.98	0.81 ± 1.20	<0.001
SBP (mm Hg)	116.25 ± 15.03	112,25 ± 20.92	0.431
DBP (mm Hg)	76.12 ± 6.75	68.69 ± 7.14	0.006

Data is presented as mean ± SD for parametric data, and median (IQ range) for non-parametric data, and p-value is also presented (paired Student's t-test or Wilcoxon test, respectively). PWL: percentage of weight loss; BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure.

Regarding the biochemical parameters of the study cohort (*Table **2***) RYGB significantly reduced blood glucose, insulin, and glycated haemoglobin (HbA1c) concentrations, which was reflected in the HOMA-IR index. At the same time, lipid parameters showed a significant decrease in levels of triglycerides, and the Atherogenic Index of Plasma (AIP), while HDL-c and Apo-A1concentrations significantly increased. However, total cholesterol and LDL-c levels did not show a statistically significant decrease.

Beyond the classical clinical markers, results were also obtained for emerging parameters. Levels of homocysteine, high-sensitivity C-reactive protein (hsCRP), and complement component C3c significantly decreased, whereas fibrinogen levels showed a slight tendency to decrease.

Table 2. Biochemistry parameters in obese patients (n=25) before and after the intervention based on bariatric surgery.

GLYCAEMIC MARKERS			
	PRE-SURGERY (Baseline)	**POST-SURGERY (12m)**	**p-value**
Glucose (mg/dl)	104.28 ± 42.44	82.8 ± 16.81	0.006
Insulin (µUl/mL)	13.57 ± 6.41	6.00 ± 2.85	<0.001
HOMA-IR	3.33 ± 1.53	1.25 ± 0.68	0<0.001
HbA1c (%)	5.30 ± 0.53	5.09 ± 0.37	0.009
LIPIDIC MARKERS			
Total cholesterol (mg/dL)	176.62 ± 42.63	170.42 ± 30.45	0.446
LDLc (mg/dL)	105.09 ± 34.10	96.64 ± 26.59	0.316
HDLc (mg/dL)	46.87 ± 13.92	57.46 ± 10.72	<0.001
Triglycerides (mg/dL)	122.46 (82.25; 122.75)	77.79 (56.25;92.75)	<0.001
Apo A1 (mg/dL)	130.56 ± 26.47	153.52 ± 27.30	<0.001
Apo B (mg/dL)	81.91 ± 27.62	78.77 ± 24.48	0.648
Apo B/ Apo A1	0.65 ± 0.24	0.53 ± 0.18	0.014
AIP	0.37 ± 0.26	0.10 ± 0.21	<0.001
EMERGENT MARKERS			
Homocysteine (µmol/L)	11.78 ± 3.35	7.87 ± 2.21	<0.001
Fibrinogen (mg/dL)	500.62 ± 109.61	454.10 ± 85.05	0.081
hs-CRP (mg/L)	13.14 (3.72; 9.34)	1.68 (0.33;2.25)	<0.001
C3c (mg/dL)	133.40 ± 21.48	105.64 ± 15.94	<0.001

Data is presented as mean SD for parametric data, and median (IQ range) for non-parametric data, and p-value is also presented (paired Student's t-test or Wilcoxon test, respectively). HOMA-IR: insulin resistance index; HbA1c: Glycated haemoglobin; LDLc: low-density lipoprotein cholesterol; HDLc: high-density lipoprotein cholesterol; ApoA1: apolipoprotein A1; ApoB: apolipoprotein B; AIP: atherogenic index of plasma; hs-CRP: high-sensitivity C-reactive protein; C3c: C3 complement.

5.2 ENDOCRINE FUNCTION ALTERATION IN ADIPOSE TISSUE IN OBESITY

The adipokines and other hormones were quantified (*Figure 6*), see more results in *Supplementary material Table 2*. Beginning with two main adipokines, leptin significantly decreased while adiponectin significantly increased. Then, the concentrations of PAI-1, significantly decreased and negatively correlated (r:-0.606; p: 0.004) with the percentage of weight loss. Also, adipsin showed a significant decrease. Leaving aside the adipose tissue, the intestinal secreted hormone, GIP significantly increased while GLP-1 didn't show significant changes.

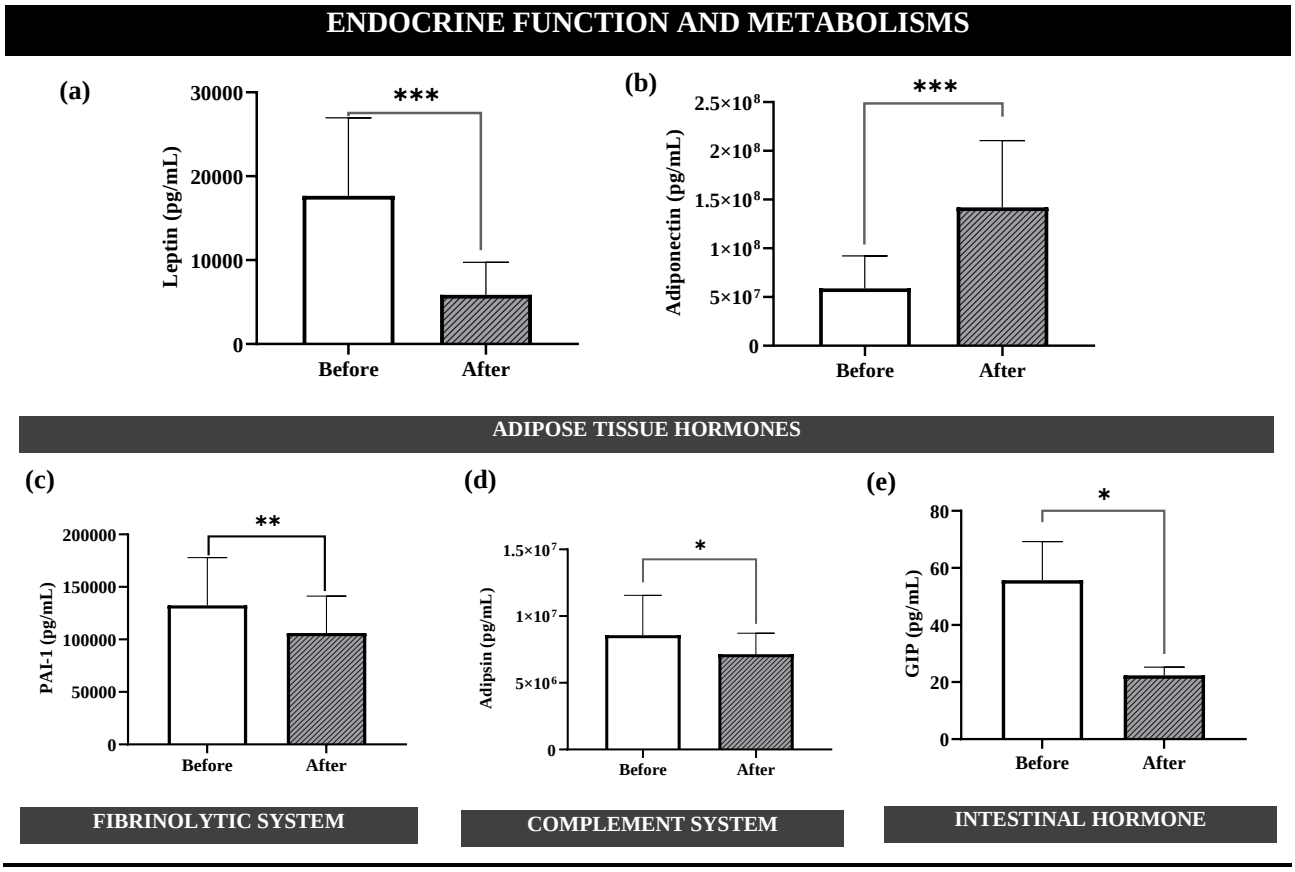

Figure 6. Metabolic hormones and regulator proteins analysed a year after the RYGB intervention. *Adipose tissue hormones:* ***a)*** *Leptin;* ***b)*** *Adiponectin. Fibrinolytic System:* ***c)*** *PAI-1 (Plasminogen Activator Inhibitor-1. Complement System: Adipsin. Intestinal hormone: GIP (Gastric Inhibitory Polypeptide or Glucose-Dependent Insulinotropic). Comparisons were made using a T-test for Leptin, Adiponectin and GIP, and Wilcoxon test for PAI-1 and Adipsin;* ****p<0,05; **p<0,01; ***p<0,001.***

5.3 SYSTEMIC INFLAMATION AND OXIDATIVE STRESS MARKERS

As shown in *Figure 7*, the pro-inflammatory cytokine IL-6 concentrations significantly decreased, while the anti-inflammatory cytokines IL-4 and IL-10 significantly increased The IL-6 values negatively correlated (r: -0,543; p: 0,011) with the percentage of weight loss. Other cytokines such as IL-8 showed a trend to increase after surgery intervention (*Supplementary material Table 2*).

With respect to oxidative stress parameters after the intervention, it was observed that patients presented non-significant changes in total ROS production (*Figure 8*). Nevertheless, mitochondrial ROS and AGEs significantly decreased one year after the intervention.

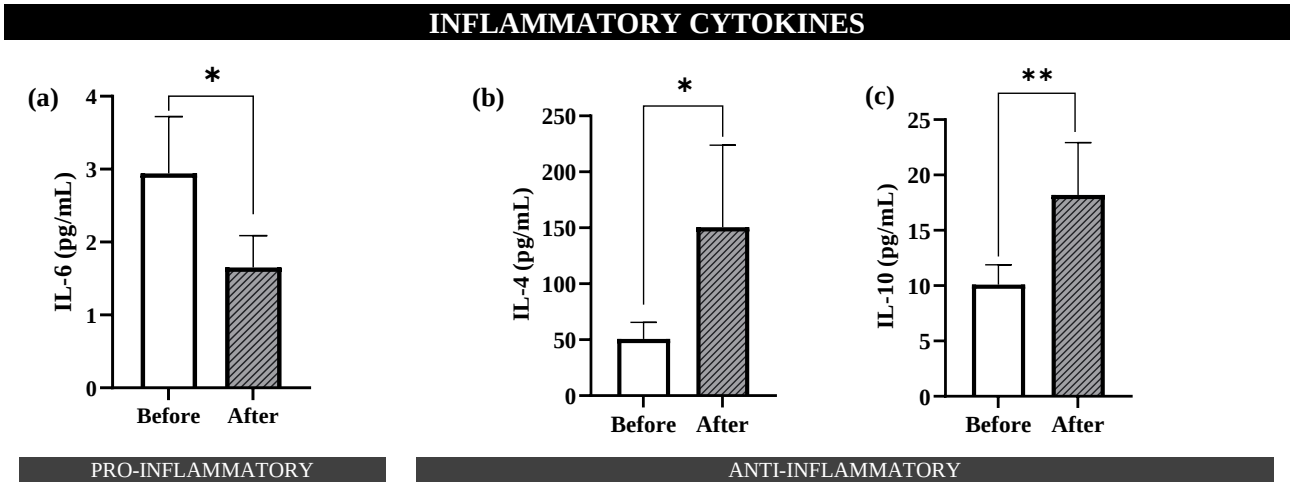

Figure 7. Inflammatory parameters in obese patients before and after the intervention based on bariatric surgery. *Pro-inflammatory cytokines:* ***(a)*** *IL-6. Anti-inflammatory cytokines:* ***(b)*** *IL-4;* ***(c)*** *IL-10. Comparisons were made using a Wilcoxon test****; *p<0,05; **p<0,01.***

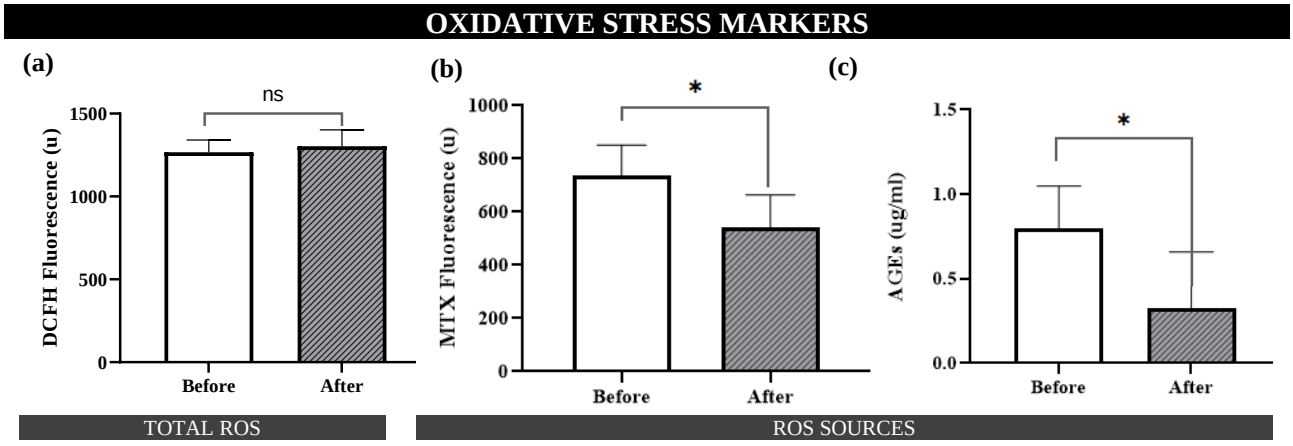

Figure 8. Oxidative stress parameters in obese patient's leukocytes before and after the intervention based on bariatric surgery. ***(a)*** *Total ROSs (DCFH);* ***(b)*** *Mitochondrial ROSs (MTX); (c) Advanced glycated end products (AGEs). Comparisons were made using a T-test;* ***ns (no significant); p< 0,05; *p<0,05.***

5.4 DNA/RNA OXIDATIVE DAMAGE

To assess the DNA/RNA oxidative damage an ELISA Assay was conducted to detect three types of oxidized guanines with the same assay (*Figure 9*). The results showed that this oxidative damage significantly decreased a year after the bariatric surgery intervention, and this levels negatively correlated (r:-0.603; p: 0.004) with the percentage of weight loss.

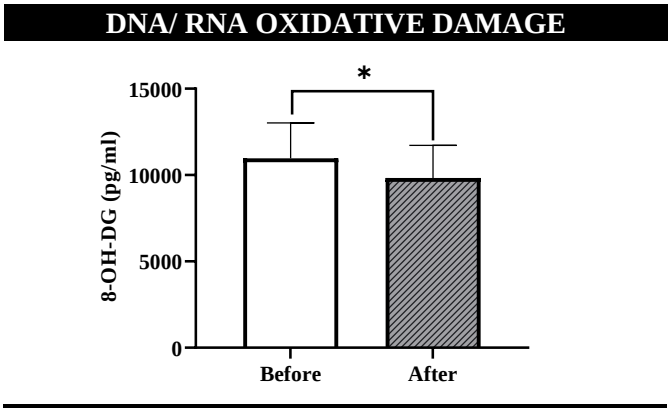

Figure 9. DNA/RNA oxidative damage quantification. *Three distinct oxidized guanine markers were analysed at the same time with an ELISA assay: 8-hydroxy-2'-deoxyguanosine (from DNA), 8-hydroxyguanosine (from RNA), and 8-hydroxyguanine (from either DNA or RNA).*

5.5 MEASUREMENT OF OXYGEN CONSUMPTION RATE

As depicted in *Figure 10*, the mitochondrial mass significantly increased. Levels of basal respiration, maximum respiration, spare respiratory capacity, ATP production, non-mitochondrial respiration, and coupling efficiency were also measured. Interestingly, the surgical intervention significantly improved basal respiration, maximum respiration, spare respiratory capacity and ATP production. However, significant differences in non-mitochondrial respiration, and coupling efficiency were not observed one year after surgery.

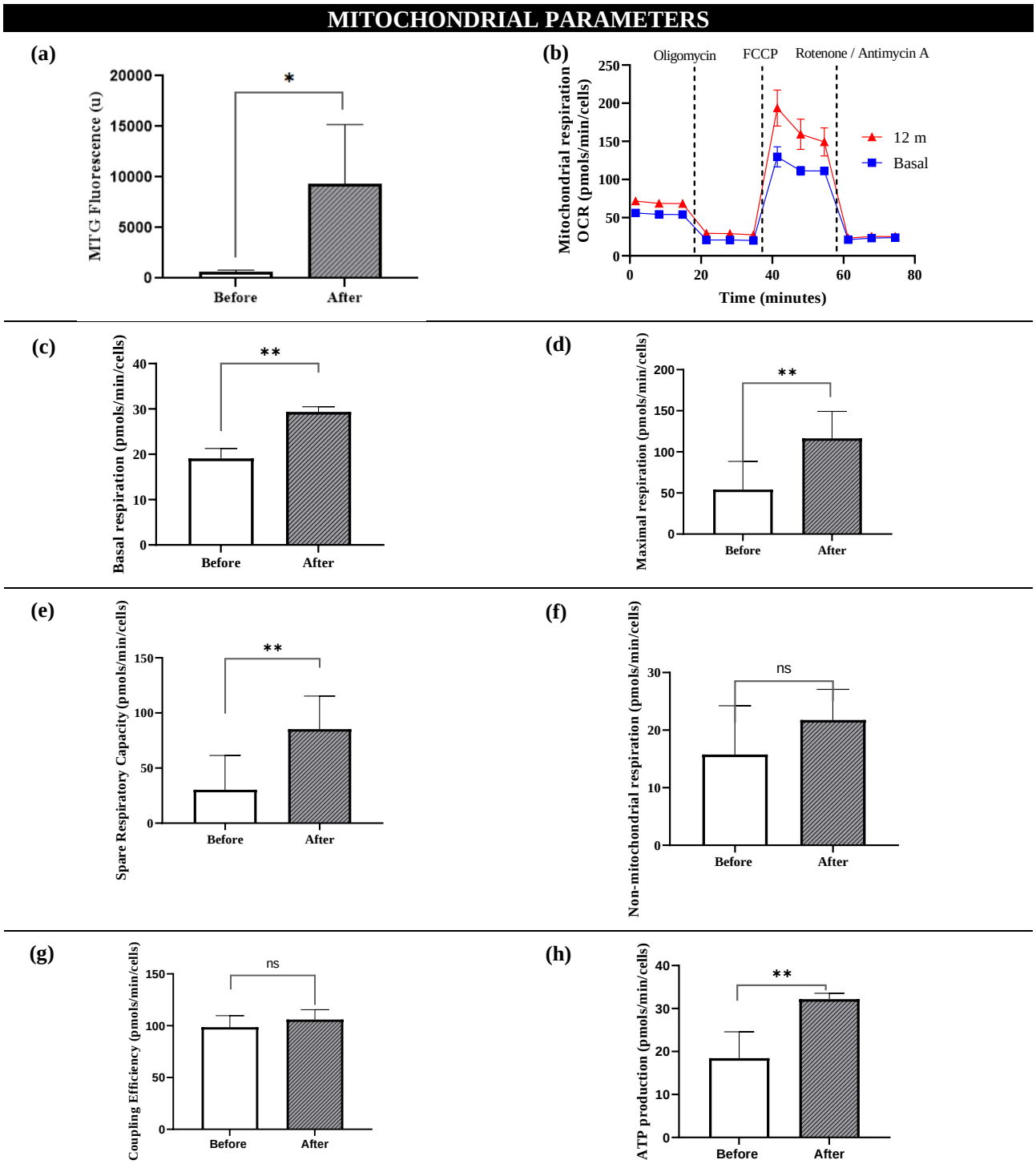

Figure 10. Mitochondrial parameters in obese patients before and after the intervention based on bariatric surgery. ***(a)*** *Mitochondrial mass in leukocytes.* ***Mitochondrial function in peripheral mononuclear cells (PBMC) from patients with obesity before and after the intervention: (b)****Patient's representative basal consumption rate during the Mito Stress test before and after the BS;* ***(c)*** *Basal respiration: oxygen consumption rate before first electronic chain inhibitor injection ;* ***(d)*** *Maximal respiration: maximal rate of respiration achieved by the cells after FCCP injection; (e) Spare respiratory capacity: mitochondria capacity to respond to energy demand;* ***(f)*** *Non-mitochondrial respiration: even though the rotenone and antimycin A injection, some enzymes continue consuming oxygen;* ***(g)*** *Coupling Efficiency: relation between ATP production rate and basal respiration rate;* ***(h)*** *ATP production: total ATP generated after the oligomycin injection. Comparisons were made using a T-test;* ***ns (no significant); p< 0,05; *p<0,05.***

6. DISCUSSION

Bariatric surgery is one of the best treatment options for extreme obesity and the comorbidities. The RYGB weight loss improved both anthropometric and biochemical parameters. Furthermore, this research has not only been conducted from a clinical standpoint but has also shown advantages in mitochondrial respiration, oxidative stress status, and metabolism regulation. The effect of RYGB on this aspect was evaluated because weight loss is a crucial component of treating obesity and can stop the emergence of several metabolic comorbidities linked to obesity.

An objective indicator of the success of the surgery in terms of weight loss and body composition changes is provided by **anthropometric parameters**. The intervention significantly reduced body weight, BMI (kg/m²), waist circumference (cm), and visceral fat values. The cohort transitioned from class II obesity (BMI 35–39.9 kg/m²) to overweight (BMI 25–29.9 kg/m²). Our findings and the existing literature report a significant post-intervention decrease in these parameters (Lin & Li, 2021a; Perdomo et al., 2023b; M. E. Piché et al., 2020). In referenced studies, similar percentage of weight loss values of 25.4, 26.9, and 26.6 were observed. It's crucial to remember that surgery doesn't result in more lean mass. Instead, the percentage of lean mass rises but its absolute value stays the same because the loss of fat mass is larger than that of lean mass.

Bariatric surgery also enhanced the **metabolism of glucose**. Improvements in HOMA-IR, insulin levels, and fasting glucose levels were noted one year after the bariatric procedure, indicating increased insulin sensitivity. A reduction in HbA1c levels further supported improved long-term glycaemic control (Zarshenas et al., n.d.). According to earlier research in the literature, these results confirm that surgery is beneficial in achieving either partial or total remission of type 2 diabetes (Perdomo et al., 2023b).

The impact on **lipid profiles** varies across studies. Although total cholesterol, LDLc, triglycerides, and apolipoproteins (Apo A1 and Apo B) values significantly improved in the majority of cases, our study and one of the reviewed studies (Perdomo et al., 2023a) did not report a significant decrease in total cholesterol at 12 months, despite a reduction being seen at 6 months. Individual factors or compliance with postoperative guidelines may have an impact on this variability. Nonetheless, a more favourable lipid profile and even a lower cardiovascular risk linked to obesity are evidently indicated by a decrease in triglycerides and an increase in HDLc values.

New biomarkers like homocysteine, fibrinogen, high-sensitivity C-reactive protein, and complement C3c can be used to evaluate how surgery affects inflammation and cardiovascular risk. The amelioration of the parameters supports a decreased risk of cardiovascular events in the future, a

decrease in fibrinogen and a reduction in low-grade chronic inflammation (Azhri et al., 2023; Zarshenas et al., 2021.).

Adipose tissue is not only an energetics storage, it's also an **endocrine tissue that produces and secretes different hormones**. The main molecules are adipokines, that promote cytokines release, to regulate many physiological actions. Adipokine secretion is altered in obesity, and can be accompanied with insulin resistance, metabolic dysregulation, inflammation and other diseases (An et al., 2023).

The Roux-en-Y gastric bypass (RYGB) effect on the endocrine function of adipose tissue was assessed by measuring the levels of classical adipokines and other metabolic regulator factors and the gut-pancreas axis at one year following surgery. Leptin and adiponectin are the adipokines more studied in the weight loss mechanism compared with the others. Weight loss from bariatric surgery significantly increased adiponectin levels and decrease leptin levels in many studies (Askarpour et al., 2020; Felipe et al., 2023; Kim et al., 2023a). In one study of patients with morbid obesity, leptin levels decreased a 74% and adiponectin levels a 64% after bariatric surgery intervention. This fact represented an 81% decrease in the leptin/adiponectin ratio. High serum triglyceride levels have been linked to elevated values of this ratio, which could indicate cardiovascular risk (Carmona-Maurici et al., 2020). Our results are consistent with the literature. As a result, bariatric surgery may help to increase leptin sensitivity and it could improve appetite control. Also, better insulin sensitivity might be associated with an increase in adiponectin, indicating a sign of a favourable metabolic profile.

Resistin is an adipokine that has received less attention. Some evidence from low-calorie diet interventions has shown that weight loss may be accompanied by a significant increase in resistin levels (Bosch-Sierra et al., 2024). Although resistin may be affected by weight loss, studies following bariatric surgery, including ours, have not reported significant changes (Askarpour et al., 2020).

Adipose tissue can also secrete molecules involved in other physiological systems. In the case of the fibrinolytic system, we found a significant reduction in PAI-1 levels following surgery. Bariatric surgery appears to reduce PAI-1 levels independently of changes in BMI, and this reduction may also be influenced by decreased triglycerides or dietary modifications (Jamialahamdi et al., 2024). These changes have already been observed as early as six months after surgery (Castro-Leyva et al., 2024) and seem to persist over time (Askarpour et al., 2020). Thus, bariatric surgery may contribute to lowering inflammation and thrombosis risk by reducing PAI-1 levels.

Regarding the complement system, adipsin plays an important role by activating the alternative pathway, which promotes C3a formation. C3a, in turn, increases insulin secretion by pancreatic beta

cells but can also promote insulin resistance. In patients undergoing gastric sleeve surgery, serum adipsin levels at six months and one year did not significantly change and did not correlate with insulin or lipid parameters (Alshubrami et al., 2020). However, in our study with RYGB patients, we observed a significant decrease in adipsin levels one year post-surgery. Further research is needed to explore the role of adipsin in obesity and its endocrine function, as to our knowledge, this is the first RYGB study to measure this parameter.

Finally, we also evaluated two hormones not secreted by adipose tissue but still relevant to appetite regulation and weight loss: GIP and GLP-1. GIP stimulates insulin release but may lose efficacy in obesity and contribute to insulin resistance. Studies have shown that GIP levels significantly decrease one year after RYGB (Kim et al., 2023a), and our findings are consistent with this. This reduction may help improve glucose regulation and reduce chronic hyperinsulinemic stimulation. As for GLP-1, levels have been shown to significantly decrease in subjects with obesity, both with normal glucose tolerance and with type 2 diabetes, after RYGB (Lindegaard et al., 2015). In our case, no significant changes were observed.

Adipose tissue dysfunction in the obese population causes the overproduction of pro-inflammatory cytokines, endoplasmic reticulum stress, activated immune cell accumulation, and reactive oxygen species (Karam et al., 2017). Because of this, tissue dysfunction is thought to be the primary cause of comorbidities linked to obesity. It is known that bariatric surgery reduces adipose tissue mass and CVD risk factors by causing improvements in the **inflammatory environment** (Turkoglu et al., 2023).

In our study, the cohort showed a reduction in percentage of fat mass and a significant increase in the serum concentration of IL-6, IL-10 and IL-4, while IL-8 showed trend to increase its serum concentration after the intervention. In fact, the percentage of weight loss negatively correlated with IL-6, and it could be related to the adiponectin levels observed, since IL-6 was reported and suggested to suppress adiponectin secretion in obesity (Lindegaard et al., 2015).

It has been difficult to compare the results with the existent literature. Mostly articles compare cytokines variations between baseline and 3-6 months after the intervention or after long-term following up (from 2 for up to 10 years). In a 2015 study (Lindegaard et al., 2015) concentrations of pro-inflammatory cytokines (IL-6, leptin and TGF-β) decreased and anti-inflammatory adiponectin increased in both obese normal glucose tolerance patients and type 2 patients. IL-8 increased 1 week and 3 months after the intervention but one year after it returned to baseline levels. A 2023 study (Kim et al., 2023b) found that BS significantly reduced IL-4 levels in diabetic patients with morbid

obesity at the first 1-month and last 12-month follow-up but did not sustain the reductions in IL-6 and TNF-β levels at the first month. Pro-inflammatory cytokines like IL-6, IL-1β, and TNF-α are typically the focus of research on alterations in circulating cytokine concentrations brought on by RYGB. A significant reduction in these markers has been consistently reported in studies that have conducted comprehensive follow-ups, both at six months and in the long term (Taylor, 2021).

The function of anti-inflammatory cytokines is still unclear, though. IL-4 and IL-10 levels significantly increased in our study, which may help to improve the inflammatory environment linked to obesity.

Obesity is associated with increased **oxidative stress** due to an overproduction of reactive oxygen species (ROS) and a decrease in antioxidant defence mechanisms. For this reason, we evaluated total ROS levels and other oxidative stress-related markers. The other sources evaluated were mitochondrial ROS and advanced glycation end-products (AGEs).

Several studies have reported improvements in oxidative stress after bariatric surgery. The intervention increased plasma antioxidant capacity and a reduction in ROS, often accompanied by a decrease in AGEs (Pradel-Mora et al., 2024). Patients with morbid obesity that undergoing bariatric surgery showed improvements in oxidative stress. Lipid peroxidation and oxidized LDL levels were reduced and antioxidant genes upregulated (Carmona-Maurici et al., 2020). In another study, in a rat model of obesity focused on liver mitochondrial dynamics, mitochondria from the bariatric surgery group showed reduced ROS levels and increased expression of genes related to mitochondrial fusion, with no significant changes in fission-related genes (Sacks et al., 2018).

In our study, we observed a significant decrease in mitochondrial ROS and AGEs after bariatric surgery, while total ROS levels remained unchanged. These results suggest that, despite the persistence of a certain degree of systemic oxidative stress, bariatric surgery is effective in reducing oxidative damage of mitochondrial origin and cumulative damage related to AGEs. Similar partial improvements have also been observed with other weight-loss strategies, such as low-calorie diets, where reductions in mtROS and increased expression of antioxidant genes occur, but total ROS levels do not significantly change (Bosch-Sierra et al., 2024).

The literature also highlights that the reduction in oxidative products may be partial and slow. For instance, some authors have noted that, despite clear metabolic improvements after surgery, subcutaneous AGE fluorescence (SAF) may remain elevated for up to five years post-surgery. This is likely due to the slow turnover of skin collagen and the long half-life of AGEs in tissue (Sánchez

et al., 2017). This suggests that while circulating AGEs may decrease more rapidly, tissue deposits require longer to clear, which may explain the only partial reduction observed in our study.

Persistent low-grade inflammation, postoperative metabolic shifts, or contributions from ROS sources other than mitochondria and AGEs could explain the lack of significant changes in total ROS levels. Overall, our findings suggest that bariatric surgery contributes to an improved oxidative stress profile. It reduced mitochondrial ROS and AGEs, even if global ROS levels remain unchanged in the short term.

Because of increased oxidative stress, cellular proliferation, and genomic instability, obesity has been linked to an increased risk of cancer (Usman & Volpi, 2018). According to studies, losing weight can reduce genomic damage. However, more investigation is required to determine how oxidative stress functions in this process. In this regard, indicators of **oxidative DNA and RNA damage** can offer important information about how bariatric surgery affects these processes.

Several studies have investigated the impact of bariatric surgery on oxidative stress markers, particularly DNA damage. In a study involving 21 patients with obesity who underwent bariatric surgery, researchers analysed the influence of weight loss on oxidative damage (Bankoglu et al., 2020). They reported a significant reduction in strand breaks and advanced protein oxidation products in peripheral blood mononuclear cells (PBMCs). The two main sources of reactive oxygen species (ROS) in these cells—mitochondrial enzymes and NADPH oxidase enzymes—remained unaltered. To assess oxidative DNA damage, they quantified 8-OHdG levels using a Cayman kit, though without specifying details. Interestingly, no significant reduction in DNA oxidation was observed. This could be attributed to the fact that the assay was performed using plasma samples from only eight patients or that only a single oxidized guanine species was analysed. Another study reported a clear decrease in oxidized cells (about a 68% reduction) and in strand breaks one year after surgery, indicating that weight reduction achieved through RYGB improves all examined oxidative stress biomarkers (Chiaramonte et al., 2023).

A similar trend was observed in another study, where 8-oxodG levels initially increased three months post-RYGB compared to preoperative values. However, this increase was temporary, as levels progressively declined throughout the follow-up period. By the two-year mark post-surgery, serum levels of 8-oxodG had decreased by approximately 12% compared to baseline. 8-oxoGuo showed a similar pattern, but the decline was more gradual starting at three months and did not include the initial spike seen for 8-oxodG (Carlsson et al., 2020).

We used the same assay to analyse three different oxidized guanine markers: 8-hydroxy-2'-deoxyguanosine (from DNA), 8-hydroxyguanosine (from RNA), and 8-hydroxyguanine (from either DNA or RNA). This was done because guanine is the most oxidation-prone DNA base and repair processes release multiple oxidized guanine species into urine. Following bariatric surgery, our results showed a significant decrease in oxidative damage to both DNA and RNA in all three species that had a negative correlation with %PWL.

This is the first study that we are aware of that measures oxidative DNA/RNA damage by measuring three distinct oxidized guanine species a year after RYGB. It is important to note that the antibody used in our assay detects multiple oxidative species, leading to higher overall levels compared to ELISA or LC-MS methods that measure a single species. Although direct comparisons of quantified values may not be possible, both our findings and existing literature consistently support the idea that oxidative DNA damage caused by obesity is significantly reduced following weight loss induced by bariatric surgery.

Alterations in the **bioenergetic profile** occur in many chronic diseases. Human mitochondria characterization under both healthy and pathological conditions, and their role in central metabolism are conducted to study this profile (ATP synthesis, ROS homeostasis, calcium buffering, and cell signaling). Characterizing mitochondrial status in obesity and the ways in which weight loss affects this profile have been the focus of numerous studies.

Obese individuals' adipose tissue exhibits decreased mitochondrial biogenesis, downregulated genes encoding elements of the mitochondrial respiratory complex, and decreased respiration/OXPHOS (Avram et al., 2022; Corkey, 2023). Given the impact of obesity on mitochondrial function, it is crucial to look into whether weight loss interventions also improve mitochondrial status. For example, in obese patients who underwent two cycles of a very low-calorie diet alternated with a low-calorie diet (24 weeks), a significant increase in basal respiration, maximal respiration, and ATP production and a reduction in mitochondrial ROS was observed (Bosch-Sierra et al., 2024). Although the increase in spare respiratory capacity and non-mitochondrial respiration was not significant.

Regarding bariatric surgery, studies have shown that in morbidly obese patients, PBMCs rapidly improve mitochondrial respiration after the procedure (Nijhawan et al., 2013). After 12 weeks, both maximal and basal respiration increased. In the long term, mitochondrial changes induced by weight loss appear to be sustained. In a cohort of patients with and without NAFLD who underwent bariatric surgery, hepatic mitochondrial respiratory capacity and hepatic mtDNA/nDNA content significantly

increased. This improvement at 12 months seems to be primarily driven by a substantial increase in mitochondrial biogenesis (Tang et al., 2024).

Based on our results, we propose that the increase in basal respiration following RYGB-induced weight loss, reflects an increase in resting energy production due to more active mitochondria, suggesting a higher energy demand under normal conditions. The increase in maximal respiratory capacity indicates that the energy production limit under high-demand conditions has expanded, allowing mitochondria to generate more energy when needed. Furthermore, the enhanced ability to adapt to high-energy demand and stress conditions (evidenced by an increase in spare respiratory capacity) reflects an improved respiratory electron-transfer pathway (ET) capacity.

Increases in ATP synthesis and mitochondrial mass also had a positive impact, improving metabolic homeostasis and energy reserve maintenance. Better mitochondrial health, avoiding the stress and oxidative damage associated with obesity, is indicated by a decrease in mitochondrial ROS. Dysfunctions in metabolism and the electron transport chain contribute to an increase in these pro-oxidant reactive species. One year after bariatric surgery, the bioenergetic profile of PBMCs had improved, enhancing their ability to meet energy demands and adapt to cellular conditions. These parameters may contribute to weight loss by regulating stress response capacity, maintaining mitochondrial homeostasis, and reducing energy stress.

It's important to remember that malabsorption, transient metabolic changes, and some acute inflammatory reactions following bariatric surgery can increase the risk of nutritional deficiencies (Yoshino et al., 2020). Results can also be greatly impacted by post-operative diet, supplement or medication use (for example lipid-lowering), and compliance with clinical follow-up.

A more complete understanding of the redox state and cellular recovery could have been assessed if limitations such as lack of endogenous antioxidant capacity values or other indicators of tissue damage, would have been performed. To determine whether the noted metabolic improvements hold up over time and to gain a better understanding of the evolution of oxidative and inflammatory markers, longer-term longitudinal studies will be necessary.

Future viewpoints ought to incorporate antioxidant defence markers (like SOD, GPx, or CAT), functional evaluations of mitochondria in particular tissues, and a more thorough description of the inflammatory profile. It would also be of interest to study subgroups of patients with and without optimal clinical response to better understand the mechanisms driving long-term effectiveness of RYGB.

7. CONCLUSION

1. After one year, RYGB surgery led to an improvement of anthropometric measurements. These indicates a remodeling of adipose tissue that led to a better glucose and lipidic metabolism regulation.

2. The endocrine function of adipose tissue changed showing a decrease of anti-inflammatory adipokines and cytokines, and an increase of anti-inflammatory molecules. All these facts suggest an amelioration of secretory profile.

3. The intervention ameliorated the chronic low-grade inflammation and the oxidative stress, preventing the amplification cycles stablished between both parameters. In addition, it induced genome stability improvement against the oxidative damage.

4. A lower intake and decreased ROS levels improved mitochondrial function. Mitochondrial metabolism increased, producing higher energy levels, enhancing biogenesis, and increasing respiratory capacity to respond to external stress.

In summary, the results of this study suggest that bariatric surgery, not only promote weight loss, but also is responsible of a complex metabolic, endocrine, and oxidative remodeling process that contributes to restoring homeostasis.

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9. SUPPLEMENTARY MATERIAL

Table 1. Supplementary information of adipokines and endocrine hormones involved in obesity development and progression. *Hormones characteristics were obtained using different sources (An et al., 2023; Hemat Jouy et al., 2024; Mancini et al., 2016; Taylor, 2021; Zorena et al., 2020).*

	Main function	Associated molecules / pathways	Obesity
Adiponectin	Anti-inflammatory, improves insulin sensitivity	Inhibits TNF-α, IL-6; inverse relation with leptin and resistin	↓
Leptin	Appetite suppression, body weight regulation	Induces IL-6; interacts with resistin and TNF-α	↑ (with resistance)
Lipocalin-2	Lipid/iron transport, pro-inflammatory	Linked to inflammation, endothelial dysfunction, insulin resistance	↑
Resistin	Promotes insulin resistance, pro-inflammatory	Activates TLR-4/NF-κB; synergistic with TNF-α, IL-6	↑
Adipsin (Factor D)	Adipocyte differentiation, activates complement alternative pathway	Acts via C3a/C3aR; interacts with PAI-1, TNF-α, β-cells	↓
Ghrelin	Appetite-stimulating (orexigenic effect)	Inhibited by leptin; stimulates NPY and dopamine	↓
GIP	Incretin: stimulates insulin secretion	Reduces IL-6 and TNF-α in adipose tissue; interacts with GLP-1	Variable
GLP-1	Incretin, enhances satiety and insulin sensitivity	Acts on CNS, pancreas, adipose tissue; upregulated by IL-6	↓ Slightly
PP	Appetite/motility regulation	Works in balance with PYY and GLP-1	No clear
PYY	Satiety signal, slows gastric emptying	Synergistic with leptin and GLP-1	↓ or altered
Insulin	Glucose regulation, anti-lipolytic	Interacts with leptin, adiponectin, ghrelin	↑ (with resistance)

Table 2. Supplementary table for adipokine metabolic hormones results.

Adipokine Metabolic Hormones: DIGESTIVE SYSTEM			
(pg/mL)	**PRE-SURGERY (Baseline)**	**POST-SURGERY (12m)**	**p-value**
Ghrelin	97,80 ± 93,81	103,85 ± 79,70	0,834
GIP	55,71 ± 66,37	22,37 ± 13,96	0,025
GLP1	138,62 ± 251,58	12,79 ± 8,17	0,085
PP	114,16 (52,49; 127,23)	154,70 (43,32; 109,34)	0,475
PYY	152,62 ±103,37	189,84 ± 127,93	0,404
Adipokine Metabolic Hormones: ADIPOSE TISSUE			
Adiponectin	58,89 ± 33,24	142,08 ± 68,51	<0,001
Lipocalin	542624,38 ± 306890,75	491185,31 ± 195033,62	0,375
Resistin	73344,62 ± 28550,80	70798,44 ± 22527,08	0,628
Adipsin	8567524,16 (6629737,55; 10054727,10)	7146837,50 (6261247,60; 8228874,51)	0,024
Leptin	17655,63 ± 9309,20	5866,11 ± 3876,32	<0,001
PAI-1	132368,74 (113231,19; 161012,10)	106148,70 (84090,54; 124754,50)	0,004
Adipokine Metabolic Hormones: INFLAMATION MARKERS			
IFNγ	43,14 ± 23,80	44,26 ± 24,49	0,657
IL-10	10,09 (4,67; 11,47)	18,19 (8,66;18,75)	0,003
IL-17	30,28 ± 21,38	31,22 ± 23,09	0,616
IL-1b	4,61 (1,92;4,83)	2,97 (1,66;4,45)	0,549
IL-2	7,70 (3,11; 6,53)	5,13 (3,07; 6,10)	0,361
IL-4	50,75 (14,45; 60,62)	150,53 (18,34;109,15)	0,028
IL-23	554,42 (223,47; 777,41)	500,82 (238,43; 622,48)	0,605
IL-6	2,94 (0,81; 3,71)	1,65 (0,52; 1,97)	0,046
IL-8	20,78 (7,28; 15,31)	27,70 (8,48; 31,86)	0,081
TNFα	19,57 (13,93; 20,76)	18,78 (14,50; 19,72)	0,710

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