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Original Article

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Bariatric Surgery Reduces Lipid Profile and Oxidative Stress in Patients With Obesity: A Prospective Cohort Study

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ABSTRACT

Purpose: To evaluate inflammatory and biochemical parameters in the serum of patients with obesity before and after bariatric surgery.
Materials and Methods: An epidemiological study of the prospective cohort type was conducted to follow patients classified with grade II or III obesity undergoing bariatric surgery. Body mass index (BMI), lipid profile, C-reactive protein (CRP), reactive oxygen species production using dichlorofluorescein (DCF), and antioxidant defenses superoxide dismutase (SOD) and reduced glutathione (GSH) were analyzed before and 3 months after Roux-en-Y bariatric surgery.
Results: A paired analysis was conducted, evaluating 23 patients in the pre- and post-surgical period. A statistically significant reduction was observed after bariatric surgery in BMI (P value<0.001), total cholesterol (CT) (P value=0.0006), total triglycerides (P value=0.0025), high-density lipoprotein cholesterol (P value=0.0010), low-density lipoprotein cholesterol (P value=0.0189), CRP (P value=0.0130), DCF (P value=0.0069), and GSH (P value<0.0001), as well as an increase in SOD activity (P value=0.0005).
Conclusion: Bariatric surgery effectively reduced inflammatory and lipid markers and reversed oxidative stress, indicating that the procedure improves the health of bariatric patients across various parameters.

Keywords: Obesity; Bariatric surgery; Lipoproteins; Inflammation mediators; Oxidative stress

INTRODUCTION

Obesity is a chronic disease of multifactorial etiology, defined as an excessive accumulation of adipose tissue, associated with low-grade systemic chronic inflammation and characterized by abnormal production of adipokines and activation of pro-inflammatory pathways. The term oxidative stress refers to an imbalance between the production of reactive species and antioxidant defenses, becoming harmful when inflammation becomes systemic, as seen in obesity. This loss of control over free radical production can lead to tissue damage.

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The consequences of oxidative stress may include damage to lipids, DNA, proteins, and carbohydrates, as well as cell death [1].

Bariatric surgery aims to achieve a reduction in body weight in cases of obesity among individuals who have been unable to sustain weight loss through non-surgical methods. This procedure is indicated only for patients with obesity, with a body mass index (BMI) above 40 kg/m^2 or a BMI above 35 kg/m^2 with associated comorbidities. It is crucial to recommend bariatric surgery considering the complications that arise with the expansion of body fat mass, altering the patient's inflammatory and metabolic profile [2].

With continuous advancements over the past 20 years, Roux-en-Y gastric surgery has commonly been regarded as the first choice. Among open procedures, the Roux-en-Y has shown positive results compared to restrictive procedures in terms of weight loss, correction of comorbidities, and long-term complications, making it the procedure of choice for many surgeons [3].

The effects of bariatric surgery include metabolic improvements, as it can trigger anti-inflammatory mechanisms and stimulate the loss of adipose tissue, which, in turn, may inhibit the expression of pro-inflammatory molecules. In these individuals, oxidative stress tends to decrease as the inflammatory state is reduced. Bariatric surgery also results in benefits to the lipid profile, facilitating the remission of post-surgical dyslipidemia and potentially involving changes in the distribution and function of visceral fat [4].

Considering that increased body weight has an inflammatory nature and that bariatric surgery can reduce weight and, consequently, body inflammation, the central aim of this study is to identify adipose tissue loss post-surgery, evaluate oxidative stress markers and antioxidant defense markers, as well as the inflammatory marker C-reactive protein (CRP) and lipid profile, assessing the improvement in the inflammatory state of patients with obesity before and after bariatric surgery.

MATERIALS AND METHODS

The study was submitted to the Research Ethics Committee and approved for data collection (approval number 6.323.640), adhering to the principles of Resolution 466/2012 of the Brazilian National Health Council. The study was approved by the Research Ethics Review Board of University of Southern Santa Catarina (UNISUL) and written informed consent was given by all participants at the time of recruitment.

An epidemiological study of the prospective cohort type was conducted to follow the patients under study. The participants underwent bariatric surgery using the Roux-en-Y gastric bypass technique at a specialized clinical hospital in Tubarão, Santa Catarina, Brazil.

For sample size calculation, the OpenEpi program was used, based on a percentage of exposed individuals with favorable outcomes of 60%, a study power of 80%, and a significance level of 95%. This yielded a sample size of 22 patients using the Fleiss method.

The study included all patients preparing for Roux-en-Y bariatric surgery who agreed to participate in the collection of information and biological samples during the first stage

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of the research, which occurred in the pre-surgical period (the week prior to surgery), and during the final stage, 3 months post-surgery. Patients who did not participate in the data collection during the final stage of the study, as well as those who did not proceed with bariatric surgery, were excluded.

Blood samples were collected for the analysis of CRP, reactive oxygen species (ROS) production through dichlorofluorescein (DCF), superoxide dismutase (SOD), reduced glutathione (GSH), and lipid profile (triglycerides [TG], total cholesterol [TC], high-density lipoprotein [HDL], low-density lipoprotein [LDL]). To conduct all the analyses, 1 mL of serum from each patient was collected in Vacutainer® vacuum collection tubes.

The analysis of ROS production was determined by the intracellular formation of DCF. Initially, the samples were homogenized, and then 2,7-dichlorodihydrofluorescein diacetate was added, followed by incubation for 30 minutes at 37°C. Fluorescence was measured using an excitation wavelength of 488 nm and an emission wavelength of 525 nm, with a calibration curve generated using standard DCF (0–10 μM). The results were expressed as fluorescence/mg of protein [5].

The analysis of antioxidant enzyme activity of SOD was determined by the inhibition of adrenaline auto-oxidation, measured spectrophotometrically, as described by Bannister and Calabrese [6]. The technique is based on the inhibition of the reaction between the $O_2\bullet^-$ radical and adrenaline, a compound that auto-oxidizes with pH variation. The oxidation of adrenaline leads to the formation of adrenochrome, and the activity of SOD was determined by measuring the rate of adrenochrome formation, spectrophotometrically at 480 nm, in a reaction medium containing glycine-NaOH (50 mM at pH 10.2) and 60 mM adrenaline. The results were expressed in U/mg of protein.

The analysis of GSH levels was determined as described by Hissin and Hilf [7]. GSH was measured after protein precipitation with 1 mL of 10% trichloroacetic acid. Phosphate buffer (800 mM, pH 7.4) and 500 μM of 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) were then added. The color development resulting from the reaction between DTNB and thiols peaked at 5 minutes and remained stable for more than 30 minutes. Absorbance was measured at 412 nm after 10 minutes. A standard GSH curve was used to calculate its levels in the samples. The results were expressed in μmol/mg of protein.

For the analysis of CRP, the CRP LATEX kit from Beckman Coulter, Inc. (Brea, CA, USA) was used through the turbidimetric method [8]. For TC analysis, the Cholesterol kit from Beckman Coulter, Inc. was used through the colorimetric method [8]. For triglyceride analysis, the Triglyceride kit from Beckman Coulter, Inc. was used with the colorimetric method, using the Beckman Coulter AU680 clinical chemistry analyzer [8]. For HDL cholesterol (HDL-C) analysis, the HDL-Cholesterol kit from Beckman Coulter, Inc. was used through the colorimetric method [8]. The calculation for LDL cholesterol (LDL-C) analysis was performed using Martin's formula [9]: LDL-C = TC – (HDL-C − TG/x).

The data were analyzed using GraphPad Prism software version 10.3.0 (GraphPad Software Inc., San Diego, CA, USA). The quantitative variables of age, body weight, and laboratory analyses were described using measures of central tendency and data dispersion. The qualitative variable of sex was described using absolute and percentage frequencies. To assess the normality of the quantitative data, the Shapiro-Wilk test was applied. When the

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distribution was normal, parametric statistics were used with Student's t-test for mean comparison. When the variable's distribution was non-normal, non-parametric statistics were applied using the Wilcoxon-Mann-Whitney U test. The statistical significance level adopted was 5% (P value<0.05).

RESULTS

A total of 23 patients were evaluated pre-intervention and the same 23 patients were assessed post-intervention. The sample consisted of 8 men and 15 women, classified with grade II or III obesity, with a mean age of 37.8 years (range: 25 to 70 years, standard deviation [SD]=10.41). Regarding body weight, the average weight of the patients pre-surgery was 112.36 kg (range: 72–185 kg, SD=29.85), and after 3 months post-surgery, it decreased to 80.25 kg (range: 52–127 kg, SD=19.6). This weight reduction was statistically significant (P value<0.001).

When comparing BMI before and after the intervention, the average decreased from 40.41 kg/m^2 (range: 33.3–59.8 kg/m^2, SD=6.95) to 28.94 kg/m^2 (range: 22.7–45.5 kg/m^2, SD=4.99), showing a statistically significant reduction from grade II and III obesity to overweight in just 3 months post-intervention (P value<0.001).

Table 1 presents the average values of tests before and after the intervention, related to complete lipid profile evaluations based on TC, TG, HDL, and LDL results. Following this, evaluations of ROS levels from DCF and the inflammatory marker CRP, as well as the antioxidant markers SOD and GSH, were assessed.

When comparing the test data performed before the surgical procedure and after 4 weeks, it was observed that all parameters significantly improved, showing statistically significant changes in all patterns related to patient health improvement. **Figs. 1** and **2** graphically present the comparisons of the parameters assessed before and after bariatric surgery, demonstrating statistically significant differences in all parameters.

Regarding the lipid profile, there was a statistically significant difference in the markers TC (P=0.0006), TG (P=0.0025), HDL (P=0.001), and LDL (P=0.0189). Similarly, regarding inflammatory and oxidative stress markers, there was also a statistically significant difference in DCF analysis (P=0.0069) and CRP (P=0.013), along with the antioxidant markers GSH (P<0.0001) and SOD (P=0.005).

Table 1. Comparison of means of biochemical, inflammatory, and oxidative stress parameters of patients pre- and post-bariatric surgery

Variables	Normal range	Pre surgery	Post surgery (3 months)	P value
Total cholesterol	<190 mg/dL	194.91±41.27	167.13±35.91	0.0006*
Triglycerides	<150 mg/dL	146.39±71.07	99.95±38.13	0.0025*
HDL cholesterol	>40 mg/dL	50.13±10.05	43.09±11.02	0.001*
LDL cholesterol	<50 mg/dL	118.60±38.39	104.18±26.89	0.0189*
2,7-dichlorofluorescein	[a]	9.91±1.74	8.10±4.55	0.0069*
C-reactive protein	<10 mg/L	10.03±13.27	5.11±7.3	0.013*
Reduced glutathione	[a]	0.60±0.18	0.50±1.09	<0.0001*
Superoxide dismutase	[a]	0.61±0.13	0.80±0.25	0.005*

Values are presented as mean ± standard deviation.
HDL = high-density lipoprotein, LDL = low-density lipoprotein.
[a]There are no normality parameters (reference values) for this dosage.
*P<0.05.

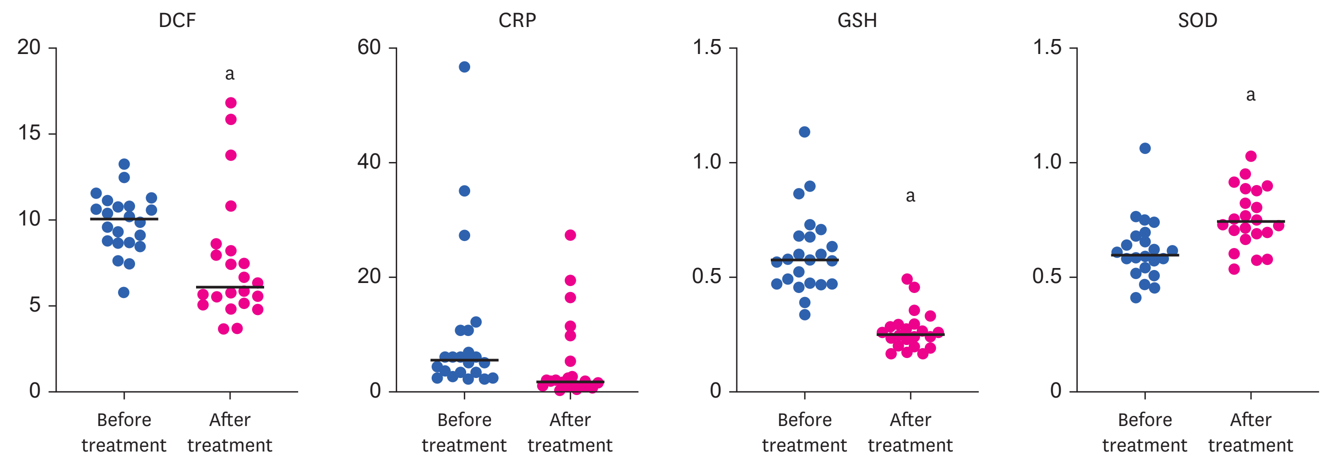

Fig. 1. A 2,7-DCF, CRP, GSH and SOD before and three months after surgery.
DCF = dichlorofluorescein, CRP = C-reactive protein, GSH = reduced glutathione, SOD = superoxide dismutase.
[a]Symbolizes a significant difference after bariatric surgery.

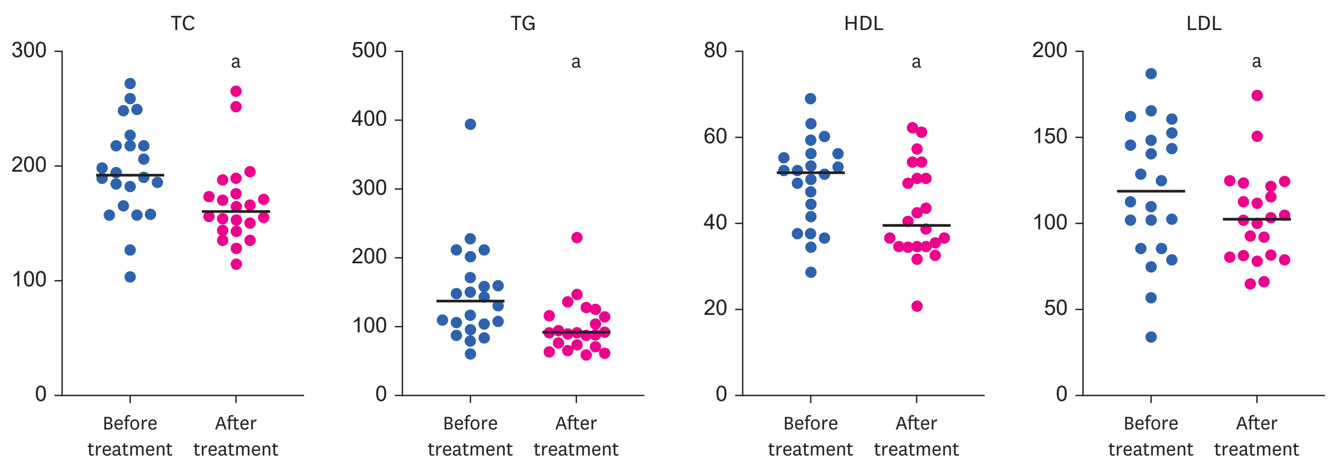

Fig. 2. Total cholesterol, triglycerides, HDL cholesterol and LDL cholesterol before and three months after surgery.
TC = total cholesterol, TG = triglycerides, HDL = high-density lipoprotein, LDL = low-density lipoprotein.
[a]Symbolizes a significant difference after bariatric surgery.

DISCUSSION

This study evaluated data related to markers of body inflammation and oxidative stress, endogenous antioxidant defenses, and lipid profile in patients undergoing bariatric surgery with the Roux-en-Y gastric bypass technique. The analyses were performed preoperatively and 3 months after the surgical procedure. Data were collected from the patients' medical records, including age, height, body weight, and BMI calculation. Lipid profile analyses were conducted, including TC, total TG, HDL-C, and LDL-C. Additionally, analyses were performed for the inflammatory marker CRP, ROS production via DCF, and antioxidant enzymes SOD and GSH.

The aim of the study was to evaluate whether the observed markers would lead to a statistically significant difference after bariatric surgery. Specifically, the study sought to assess reductions in body weight and chronic inflammation associated with obesity, improvements in overall health, and reductions in comorbidities to enhance patients' quality of life. All markers were analyzed before and 3 months after the surgical procedure, and statistically significant differences were found in all the evaluated parameters. Below, we discuss each marker individually.

The body weight of patients significantly reduced after surgery. Other studies have already demonstrated similar results. Min et al. [10] showed significant weight loss in 19 participants at 1 month, 6 months, and 4 years after undergoing bariatric surgery with different techniques. Another study with 87 participants with obesity also demonstrated a reduction in BMI after 3 years of post-bariatric surgery follow-up [11]. Finally, another study with 37 patients with obesity showed that BMI decreased at 1- and 6-months post-surgery [12]. Regarding body weight reduction, the decrease in this marker is well established in the literature after bariatric surgery with the Roux-en-Y technique [10-12].

The mechanisms that facilitate the remission of dyslipidemia after bariatric surgery may involve changes in the distribution and function of adipose tissue, improvement in insulin sensitivity, and positive outcomes in hepatic lipoprotein metabolism. Furthermore, the procedure may influence the secretion of intestinal hormones and inflammatory markers, changes in the release of adipokines and hormones, which in turn can favor lipid profile and metabolism [10-12].

Regarding TC, there was a statistically significant reduction after the surgery. A study involving 100 patients divided into 2 groups, with 50 patients undergoing Roux-en-Y surgery and 50 patients undergoing vertical sleeve gastrectomy, found that at 5 years post-surgery, TC and LDL levels decreased significantly in the Roux-en-Y group, while no differences were observed in the gastrectomy group [13].

Another study aimed at evaluating the short-term (3 months) and long-term (12 months) effects of Roux-en-Y surgery showed that although serum TC concentrations were elevated preoperatively, they decreased significantly 12 months post-surgery [14]. Arnáiz et al. [15] analyzed the variation in lipid parameters over the 3 years following surgery and observed a significant decrease in TC during all follow-up periods.

Obesity-related dyslipidemia, characterized by low HDL-C levels, hypertriglyceridemia, and small, dense LDL particles, is partially responsible for the high residual cardiovascular risk associated with this clinical condition. Regarding triglyceride levels, a statistically significant reduction was observed after surgery in the present study. A retrospective, descriptive cohort study, conducted through database consultation, included 351 patients who underwent bariatric surgery. After 6 months, significant weight loss and triglyceride reduction were observed, with a statistically significant difference [16].

Voglino et al. [17] reviewed data from 42 patients who underwent laparoscopic sleeve gastrectomy and 61 patients who underwent Roux-en-Y gastric bypass. In the Roux-en-Y group, patients showed significant improvement in all lipid parameters evaluated over time, while patients with sleeve gastrectomy only experienced a reduction in triglyceride levels and an increase in HDL-C. Zaki et al. [18] used a retrospective approach to analyze the lipid profile of patients who underwent bariatric surgery with the sleeve technique. Data from 163 patients showed a significant reduction in triglyceride levels.

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The levels of HDL were also statistically reduced when compared to the pre-surgical period. Garay et al. [13] observed increased HDL levels in both Roux-en-Y (62.69±16.3 mg/dL) and vertical sleeve gastrectomy (60.64±18.73 mg/dL) procedures, with no significant difference between the procedures. Another study demonstrated bariatric surgery's ability to significantly raise HDL levels in patients [18]. A review showed that in both Roux-en-Y and sleeve gastrectomy procedures, patients experienced a significant increase in total HDL 1 year after the surgery [17]. Pedron et al. [16] also demonstrated increased total HDL levels at 6 months, 12 months, and 24 months post-surgery.

Our study, unlike previous studies, showed a reduction in HDL levels, possibly related to the decrease in TC levels observed after bariatric surgery. Human trials suggest that within the first 6 months during rapid weight loss with bariatric surgery, HDL decrease, our data suggest a significant alteration in HDL structure that is accompanied by an increase in its antioxidant potential [19].

The initial drop in HDL-C levels may reflect the gradual qualitative switch in HDL particles from Apo E-containing to more functional Apo A1-containing HDL particles, which may explain the improvement in HDL structure and functionality. Another theory, is that the decrease of HDL may also be associated with the potential sedentary behavior of patients after surgery, as HDL-mediated cholesterol transport levels are elevated with regular physical activity [19].

Finally, the results related to the LDL evaluation also showed a statistically significant reduction compared to the pre-surgical period. Guzel and Ikizek [14] measured lipid changes at 3 and 12 months after bariatric surgery and demonstrated that although serum LDL-C concentrations were elevated preoperatively, they significantly decreased 12 months post-surgery. In another study, a significant decrease in LDL and non-HDL-C was observed with the biliopancreatic diversion technique [15].

Genua et al. [20] collected plasma samples before surgery, as well as at 6 and 12 months after the intervention, from 13 patients who underwent bariatric surgery. The atherogenic properties of LDL and HDL were altered in individuals with obesity compared to the controls, with smaller LDL particles that are more susceptible to modification and smaller HDL particles with reduced antioxidant capacity. Bariatric surgery normalized the composition of lipoproteins and improved the qualitative characteristics of both LDL and HDL.

Regarding the lipid profile, bariatric surgery results in benefits for lipoprotein profiles and metabolism through multiple mechanisms. It facilitates the remission of dyslipidemia and involves changes in the distribution and function of visceral fat, improves insulin sensitivity, and shows positive outcomes in hepatic adipose content [21].

The improvement in the lipid profile may lead to a reduction in body inflammation. In this regard, the inflammation measured in our analysis using CRP showed a statistically significant reduction in the post-surgical period. A prospective study with 19 participants who underwent different bariatric surgery techniques demonstrated that at 1 month, 6 months, and 4 years, all analyses showed a statistically significant reduction in CRP levels [10].

In another study with 87 participants with obesity, followed for a period of 3 years, a reduction in the inflammatory marker was also demonstrated [11]. Chiappe et al. [12]

analyzed 37 patients with obesity and conducted evaluations before, 1 month, and 6 months after surgery, where high-sensitivity CRP levels decreased significantly after 6 months.

In general, serum levels of CRP decrease after bariatric surgery, correlating with the loss of adipose tissue. This occurs because the inflammatory state can change rapidly due to strong regulation in cytokine production, triggering anti-inflammatory mechanisms and stimulating weight loss, which in turn can inhibit the expression of pro-inflammatory molecules [4].

In addition to chronic inflammation, obesity can lead to an increase in reactive oxygen and nitrogen species, intensifying cellular damage and promoting the progression of adverse metabolic conditions [22]. In this sense, the values related to the analysis of ROS production, determined by DCF, showed a statistically significant reduction in the post-surgical period compared to the pre-surgical period.

A study with 35 patients with obesity evaluating the relationship between antioxidant and oxidant balance (PAB) 6 months after Roux-en-Y gastric bypass surgery showed a significant post-operative reduction in serum PAB values compared to baseline [23]. Pradel-Mora et al. [22] conducted a systematic review, identifying 30 studies and analyzing the connection between oxidative stress and recovery after bariatric surgery. The results revealed a significant decrease in oxidative stress biomarkers post-surgery.

During obesity, disturbances in lipoprotein metabolism and glucose homeostasis drive pro-inflammatory activation and the differentiation of macrophages and other immune cells. These responses result from oxidative stress production, changes in the cell's redox potential, and the overexpression of key regulators of inflammation [24].

The mechanisms associated with oxidative stress in patients with obesity arise from the excessive accumulation of adipose tissue, leading to a chronic inflammatory state due to the increased release of pro-inflammatory adipokines, hyperglycemia, and a reduction in antioxidant defenses. After bariatric surgery, there is a reduction in ROS levels as the inflammatory state decreases due to fat loss and improved insulin sensitivity [25].

In addition to the reduction of oxidative agents after bariatric surgery, the antioxidant defense marker SOD showed a statistically significant increase in its activity when comparing pre- and post-surgical intervention. A study with 57 individuals with obesity who underwent Y-roux surgery demonstrated an increase in SOD antioxidant activity and a significant reduction in the number of carbonyl groups in serum proteins [26].

On the other hand, a study that included 65 patients with obesity, evaluating markers at 1, 3, 6, and 12 months after bariatric treatment, showed that serum SOD activity was significantly decreased at all periods following surgery [27]. Pinto et al. [28] assessed changes in oxidative stress in patients undergoing Y-roux surgery 3- and 12-months post-surgery, where the SOD enzyme decreased at both 3 and 12 months compared to the pre-surgery period. These findings suggest that although bariatric surgery contributes to improvements in redox homeostasis, the effects of chronic obesity may perpetuate the role of adipose tissue as a significant source of ROS and inflammation, even after weight loss.

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Indeed, a decrease in antioxidant enzyme activity is commonly observed in patients with obesity, leading to the accumulation of ROS, which cause damage to proteins, lipids, and DNA [29]. On the other hand, in the present study, following bariatric surgery and the subsequent weight loss, hydrogen peroxide (H_2O_2) levels significantly decreased, along with an increase in SOD activity. This increase may be a result of improved antioxidant capacity in these patients, leading to a reduction in the production of ROS.

However, as observed in other studies [26,27], SOD activity can vary in patients with obesity. These differences in antioxidant enzyme activity may be explained by factors such as age, sex, severity and duration of the condition, and the types of surgical procedures performed.

The values regarding the analysis of GSH levels in our study showed a statistically significant reduction in the post-surgical period compared to the pre-surgical period. Another study also demonstrated that GSH concentration was significantly lower in the plasma of the obesity group before bariatric surgery compared to the control group. Moreover, GSH concentration was significantly reduced in the plasma of patients with obesity 3 and 6 months after bariatric treatment [27].

In our study, an increase in SOD levels was observed. SOD is an enzyme that catalyzes the conversion of superoxide into hydrogen peroxide, serving as a mechanism to neutralize superoxide, making it less reactive or directing it toward water production. With the increase in SOD enzyme levels, antioxidant efficiency occurs, scavenging superoxide to mitigate cellular damage and initiating the process of neutralizing ROS [30].

When determining hydrogen peroxide levels, lower levels are observed, as DCF levels are decreasing even with the activation of the SOD enzyme. This occurs because the body is consuming glutathione, as indicated by the reduction in GSH levels. With reduced GSH levels, glutathione peroxidase (GPx) activity is activated since GSH is one of its substrates. The antioxidant enzyme GPx converts hydrogen peroxide into water, creating a positive impact by removing ROS from the body and preventing excessive production, which would otherwise lead to increased oxidative stress [30].

However, the present study has some limitations, including the number of patients evaluated and the time between pre- and post-intervention assessments, as well as sample losses due to the discontinuation of some patients, in addition to the geographical limitations of the study (Brazilian cohort). In addition, even with a reduced sample size, the results obtained allowed us to confirm that treatments offering alternatives for individuals with excess weight and seeking a better quality of life are important and necessary for managing this increasingly common condition in society today.

This research reinforces the relevance of treatments in cases where excess weight can no longer be addressed solely through conventional approaches, such as lifestyle changes, but rather by considering various contexts and related factors. By contributing to the understanding of the benefits of this practice for quality of life, this study highlights the need for implementing interventions that promote the health and well-being of patients diagnosed with obesity.

On the other hand, metabolic and inflammatory changes typically require long-term observation, a 3-month follow-up may not fully capture the sustained effects. That being

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said, longer follow-up studies are necessary to assess whether these metabolic improvements persist over time.

CONCLUSION

In conclusion, bariatric surgery using the Roux-en-Y technique was able to promote positive changes in BMI indices through body weight reduction and improvements in lipid profile parameters (TC, TG, HDL-C, and LDL-C), as expected by the study participants. Furthermore, there was a statistically significant reduction in inflammatory parameters, as observed through CRP levels, and a decrease in oxidative stress, as indicated by the reduced production of ROS, activation of SOD, and reduction of GSH.

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