

Original article

## Effects of omentectomy in addition to sleeve gastrectomy on the metabolic and inflammatory profiles of obese rats

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### Abstract

**Background:** Visceral obesity has been considered a risk factor for metabolic and cardiovascular complications. In an attempt to reduce the visceral adipose tissue, omentectomy has been proposed to be performed along with bariatric surgery.

**Objective:** The goal of this study was to evaluate whether omentectomy associated with sleeve gastrectomy (SG) is beneficial to the inflammatory and metabolic profile of rats fed a standard diet (STD) or high-fat diet (HFD).

**Setting:** University hospital, Brazil.

**Methods:** For this experiment, male Wistar rats were randomly divided into 6 groups as follows: sham surgery (STD+L or HFD+L), SG alone (STD+SG or HFD+SG), or SG with omentectomy (STD+SGO or HFD+SGO). Anthropometric data and metabolic profiles were evaluated, and the tissue expression of inflammatory markers in the visceral adipose tissue was measured.

**Results:** In rats with diet-induced obesity treated with SG with or without omentectomy, there was a reduction in weight (HFD+SG:  $P < .01$  and HFD+SGO:  $P < .05$ ), adiposity (HFD+SG:  $P < .001$  and HFD+SGO:  $P < .05$ ), plasma levels of glucose (HFD+SG:  $P < .01$  and HFD+SGO:  $P < .01$ ), plasma levels of C-peptide (HFD+SG:  $P < .01$  and HFD+SGO:  $P < .001$ ), plasma levels of insulin (HFD+SG:  $P < .05$  and HFD+SGO:  $P < .001$ ), plasma levels of total cholesterol (HFD+SG:  $P < .01$  and HFD+SGO:  $P < .01$ ), and tissue expression of TNF- $\alpha$  (HFD+SG:  $P < .001$  and HFD+SGO:  $P < .01$ ), but there was no statistically significant difference between the groups in which omentectomy was performed or was not.

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**Conclusion:** In this study, we did not observe additional beneficial effects due to omentectomy associated with SG in the metabolic profile and tissue expression of inflammatory markers. (Surg Obes Relat Dis 2016;12:1292–1299.) © 2016 American Society for Metabolic and Bariatric Surgery. All rights reserved.

**Keywords:** Obesity; bariatric surgery; Sleeve gastrectomy; Omentectomy; Weight loss; Inflammation markers

The adipose tissue is a complex organ with endocrine and metabolic activities, from which several factors with local (autocrine and paracrine) and systemic (endocrine) functions are secreted [1]. Obesity is associated with a chronic inflammatory process characterized by the abnormal production of proinflammatory cytokines that activate different signaling pathways. Therefore, the excess of adipose tissue, especially visceral adipose tissue, is correlated with the development of insulin resistance, glucose intolerance, dyslipidemia, hypertension, and a prothrombotic state [2–4]. Thus, resection of the great omentum has been proposed during bariatric surgery to improve the metabolic profile and to maximize the weight loss in obese patients [5]. However, the omentum possesses healing factors (growth, angiogenesis, chemotactic factors, and progenitor cells), and it promotes healing and regeneration of injured tissues when activated [6]. These important properties of the omentum allow it block intraperitoneal inflammatory processes and adhere to it, thereby preventing the development of diffuse peritonitis [7–9].

The inclusion of omentectomy as a part of the bariatric surgery technique remains controversial and questionable. Authors have shown improvement in glycemic homeostasis and the lipid profile when omentectomy is included in the bariatric surgery technique [5,10]. However, other authors have shown that the reduction of visceral adipose tissue achieved by omentectomy is not a useful approach in improving insulin sensitivity and reduction of the cardio-metabolic risk factors associated with obesity or type 2 diabetes (T2D) [11,12].

The goal of this study was to determine whether omentectomy associated with sleeve gastrectomy (SG) provides additional beneficial effects to the glycemic profile, lipid profile, and the expression of inflammatory markers in the white adipose tissue of Wistar rats fed a standard diet (STD) or high-fat diet (HFD).

## Materials and methods

### Animals and diets

Male Wistar rats aged 8 weeks were used for this experiment, and they were randomly divided into 6 groups (10 animals/group), maintained in individual cages, and exposed to a light cycle 12 h/12 h in a temperature of  $22 \pm 2^\circ\text{C}$ . The rats were treated with STD or HFD for 8 weeks and given ad libitum access to food and water. The

STD (Labina; Purina, Wilkes-Barre, PA) is composed of 50.3% carbohydrate, 41.9% protein, and 7.8% fat with a total of 2.18 kcal/1 g of diet [13]. The HFD is composed of 24.5% carbohydrate, 14.4% protein, and 60.9% fat with a total of 5.28 kcal/1 g of diet [13]. All of the HFD components were purchased from Rhoister LTDA (São Paulo, Brazil). After this period, the animals were submitted to surgical treatment as follows: simulated laparotomy (L) (sham surgery), sleeve gastrectomy (SG), and sleeve gastrectomy with omentectomy (SGO). After the second day of the postoperative period, the animals received their respective diets for 4 additional weeks. This study was approved by the Ethics Committee of Experimentation and Animal Welfare of UNIMONTES, Montes Claros, Brazil.

### Surgical procedures

The animals were submitted to anesthesia (ketamine and xylazine) administered intraperitoneally after intramuscular application of ceftriaxone for antibiotic prophylaxis. In 2 groups, only the L was performed with the manipulation of the stomach (STD+L and HFD+L). In another 2 groups, SG was performed (STD+SG and HFD+SG). In the last 2 groups, the omentectomy procedure (STD+SGO and HFD+SGO) was performed in addition to the SG. The SG was performed with an 80% resection of the stomach, including complete removal of the gastric fundus and confection of a new gastric tube by manual suture extending from the Hiss angle to the distal portion of the antrum (Figs. 1A–D). For the groups that were subjected to omentectomy, a complete dissection and resection of the whole greater omentum along the greater curvature of the stomach until the duodenum as well as the transverse colon until the spleen was performed (Figs. 2E and F).

### Weight, food intake, and tissue collection

The weight, food intake (g/BW), and energy intake (kcal/g BW) were assessed 3 times a week during the pre- and postoperative period. The animals were sacrificed by decapitation with guillotine, and blood samples were collected and centrifuged (3200 rpm for 10 min) for posterior plasma dosages. The following tissues were collected: white adipose tissue (periepididymal, retroperitoneal, and mesenteric) and brown adipose tissue (interscapular). The tissues were weighed, immediately frozen in liquid nitrogen, and stored at  $-80^\circ\text{C}$  for posterior analysis.

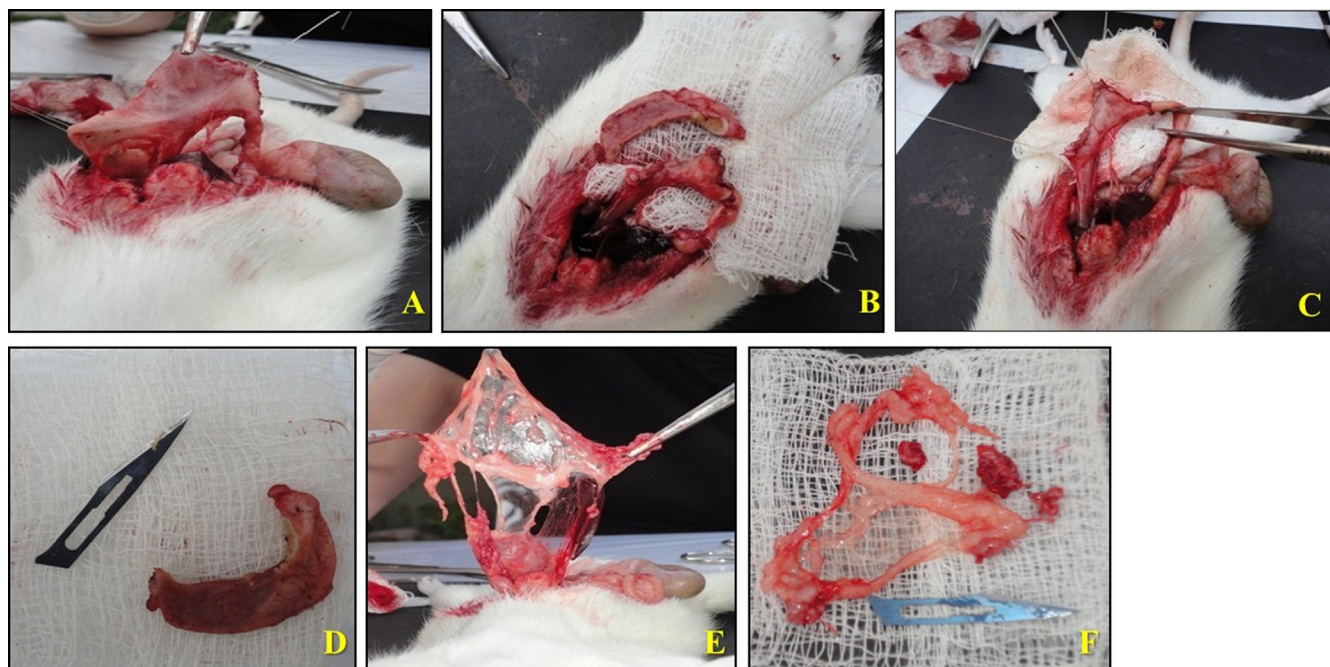


Fig. 1. Sleeve gastrectomy and omentectomy. (A) Exposition and repair of the stomach at the Hiss angle and distal antrum. (B) From 70–80% resection of the stomach including the gastric fundus. (C) Formation of the gastric tube (sleeve gastrectomy performed). (D) Stomach resected. (E) Exposition of the greater omentum. (F) Omentectomy performed.

#### *In vivo experiments: Insulin sensitivity test and glucose tolerance test*

One week before the surgery and 1 week before sacrifice, the insulin sensitivity test was performed with blood tail samples collected from the animals to determine blood glucose levels at 0, 15, 30, and 60 minutes after intraperitoneal injection of insulin (0.75 U/kg BW; Sigma, St. Louis, MO) [14]. The glucose tolerance test assessed the levels of glucose at 0, 15, 30, 60, and 120 minutes after intraperitoneal injection of glucose (2 g/kg BW) after a fasting period of 12 hours using an Accu-Check (Roche Diagnostics Corp., Indianapolis, IN) [14].

#### *Laboratory tests*

Plasma dosages of glucose, C-peptide, insulin, total cholesterol, high-density lipoprotein, and triglycerides were measured using specific ELISA kits (DSA BioELISA, USA).

#### *Reverse transcription and quantitative real time-polymerase chain reaction*

Total RNA from the periepididymal adipose tissue was prepared using TRIzol reagent (Invitrogen Corp., San Diego, CA), treated with DNase, and reverse transcribed with Moloney murine leukemia virus (Invitrogen Corp.) using random hexamer primers. Levels of tumor necrosis factor (TNF- $\alpha$ ) and interleukin (IL-6) were determined by quantitative real time-polymerase chain reaction using SYBR Green reagent (Applied Biosystems, Grand Island,

NY), the QuantStudio 6 Flex Real-Time PCR System (Applied Biosystems) and the following primers: TNF- $\alpha$  FW, 5'-ATG GGC TCC CTC TCA TCA GT-3'; TNF- $\alpha$  RV, 5'-GCT TGG TGG TTT GCT ACG AC-3'; IL-6 FW, 5'-GTC AAC TCC ATC TGC CCT TCA-3'; and IL-6 RV, 5'-GAA GGC AAC TGG CTG GAA GT-3'. Gene expression was normalized to endogenous Beta-actin (FW, 5'-TGA CAG GAT ACA GAA GGA GA-3'; and RV, 5'-TAG AGC CAC CAA TCC ACA CA-3'). The relative comparative CT method of Livak and Schmittgen was applied to compare gene expression levels between groups using the following equation:  $2^{-[\Delta\Delta C_T]}$  [15].

#### *Statistical analysis*

All data were transferred to GraphPad Prism software, version 6.0 (San Diego, CA) and analyzed using a 95% confidence level ( $P < .05$ ). Data are expressed as the mean  $\pm$  standard deviation (SD). The differences between 2 groups were analyzed by Student's *t* test. Statistically significant differences among  $>2$  groups were analyzed by 1-way analysis of variance followed by Bonferroni post hoc test.

#### **Results**

In the preoperative period, the food intake (g/BW) was higher in the STD group than in the HFD group, but the energy intake (kcal/g BW) was higher in the HFD group than the STD group, resulting in a statistically significant weight increase in all groups fed an HFD compared to the groups fed an STD (Figs. 2A–C). In the postoperative

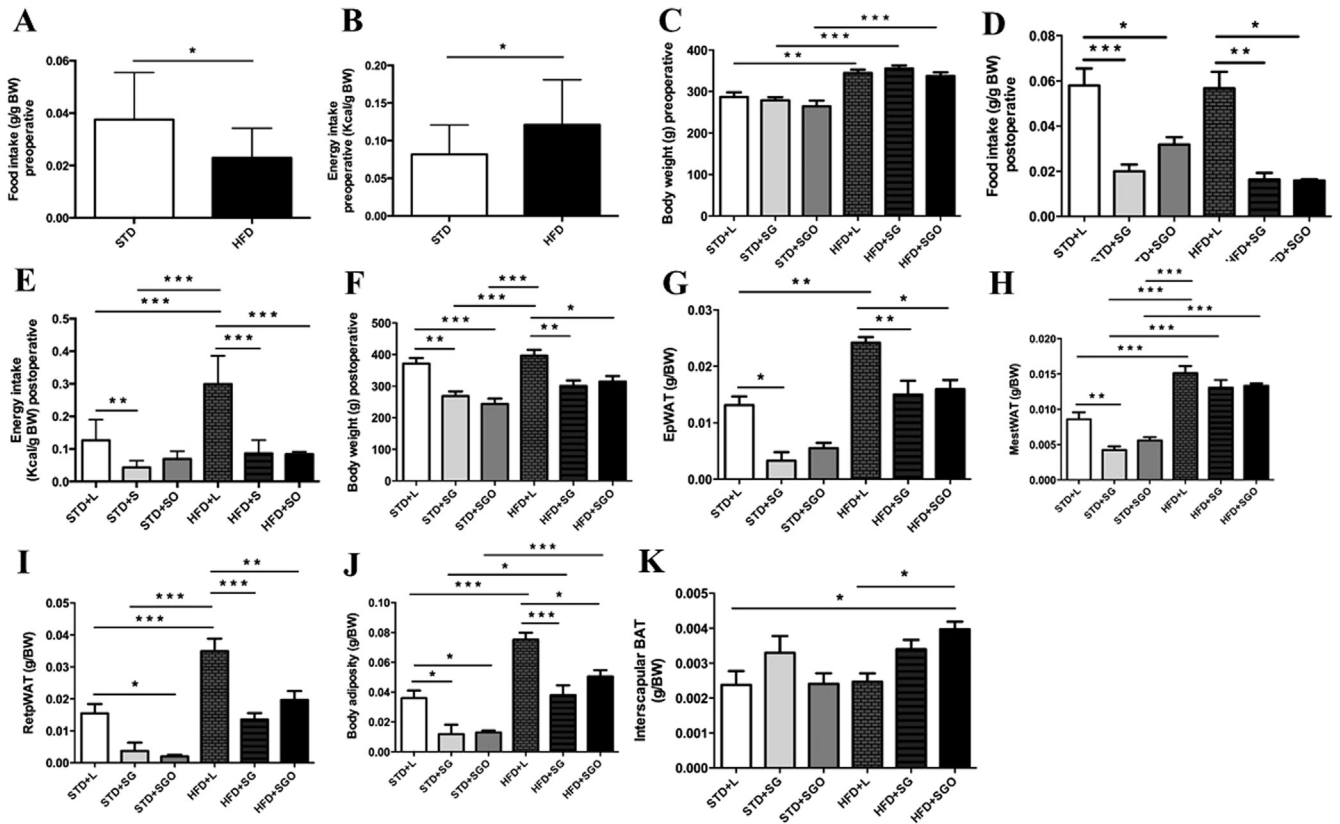


Fig. 2. Food intake, energy intake, weight, and tissue weight of rats fed a standard diet (STD) or high-fat diet (HFD) and subjected to sham surgery (L) (STD +L or HFD+L), sleeve gastrectomy (STD+SG or HFD+SG), or sleeve gastrectomy + omentectomy (STD+SGO or HFD+SGO). (A) Preoperative food intake (g/BW). (B) Preoperative energy intake (kcal/g BW). (C) Preoperative weight (g). (D) Postoperative food intake (g/BW). (E) Postoperative energy intake (kcal/g BW). (F) Postoperative weight (g). (G) Periepididymal adipose tissue weight (g/BW). (H) Mesenteric adipose tissue weight (g/BW). (I) Retroperitoneal adipose tissue weight (g/BW). (J) Body adiposity/white adipose tissue weight (periepididymal, mesenteric, and retroperitoneal tissues) (g/BW). (K) Interscapular brown adipose tissue weight (g/BW). \* $P < .05$ ; \*\* $P < .01$ ; \*\*\* $P < .001$  (Student's  $t$  tests, 1-way ANOVA, and Bonferroni posttest).

period, a significant decrease in the food intake, energy intake, weight, and adiposity (evaluated in the periepididymal, mesenteric, and retroperitoneal adipose tissues) for the animals subjected to SG was observed compared with the other groups, but no statistically significant difference was observed (Figs. 2D–J). A significant increase in the brown adipose tissue (interscapular) in the HFD+SGO group was found compared with its respective control group (Fig. 2K).

Regarding the glycemic profile, the insulin sensitivity test and glucose tolerance test performed in the preoperative period found significantly elevated glucose levels in the animals fed an HFD compared with animals fed an STD (Figs. 3A and B). The same tests performed in the fourth week of the postoperative period found a reduction in the glucose levels in the groups subjected to SG or SGO, but no significant difference among the groups fed the same diet was found (Figs. 3C and D). Similarly, the plasma glucose level (blood collected after sacrifice) found significantly reduced values in the HFD+SG and HFD+SGO groups compared with the other groups (Fig. 3E). The C-peptide and insulin dosages found reduced levels in the animals

subjected to omentectomy, but no significant difference in these dosages was observed when comparing these animals to those without omentectomy (Figs. 3F and G).

A significant reduction of the total cholesterol in the groups treated with HFD and subjected to SG was observed, and the total cholesterol values were similar in animals with or without omentectomy (Fig. 3H). In these same groups, there were no differences regarding the plasma levels of HDL and triglycerides as shown in Figs. 3I and J.

The gene expression of the TNF- $\alpha$  inflammatory marker was significantly lower in the groups treated with HFD and subjected to SG compared with the control group, but no significant difference was observed between the groups with or without omentectomy (Fig. 4A). In contrast, IL-6 expression was significantly lower in the HFD+SG group, but the same was not observed for the HFD+SGO group (Fig. 4B).

## Discussion

In this study, the effects of omentectomy associated with SG on the weight loss, glycemic profile, lipid profile, and

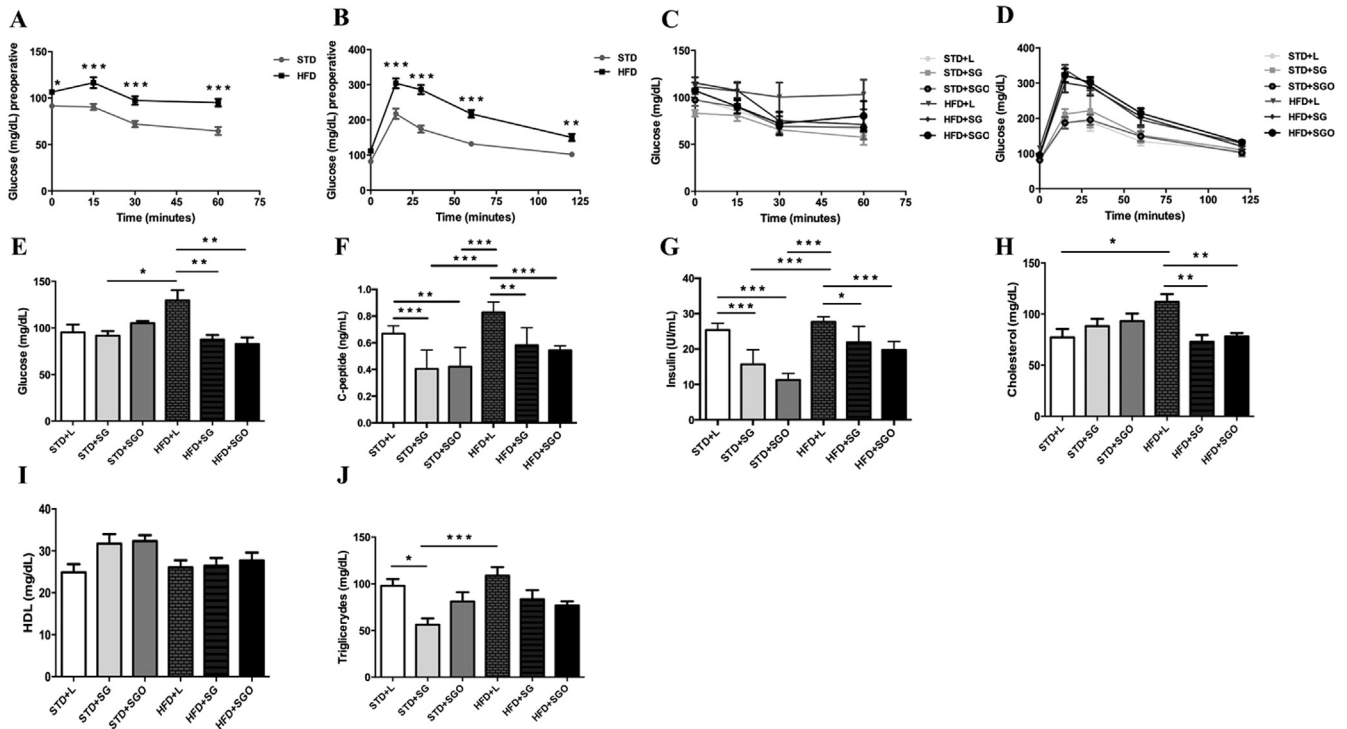


Fig. 3. Preoperative and presacrifice insulin sensitivity tests (ISTs), glucose tolerance tests (GTTs), and blood parameters of rats fed a standard diet (STD) or high-fat diet (HFD) and subjected to sham surgery (STD+L or HFD+L), sleeve gastrectomy (STD+SG or HFD+SG), or sleeve gastrectomy + omentectomy (STD+SGO or HFD+SGO). (A) Preoperative IST (mg/dL). (B) Preoperative GTT (mg/dL). (C) Presacrifice IST (mg/dL). (D) Presacrifice GTT (mg/dL). (E) Plasma glucose (mg/dL). (F) Plasma C-peptide (ng/mL). (G) Plasma insulin (UI/mL). (H) Total cholesterol (mg/dL). (I) HDL (mg/dL). (J) Triglycerides (mg/dL). \* $P < .05$ ; \*\* $P < .01$ ; \*\*\* $P < .001$  (1-way ANOVA and Bonferroni posttest).

gene expression of inflammatory markers in the white adipose tissue of male Wistar rats fed an STD and HFD-induced obesity were evaluated. There is evidence that this animal model is appropriate to study the induction of obesity and the performance of SG with hand-sewn or suture stapling techniques as well as the posterior evaluation of the corporal, biochemical, and molecular profiles

[16–18]. In the preoperative period, a significant increase in the weight of animals fed an HFD was observed. In the postoperative period, however, a reduction in food intake (g/BW), energy intake (kcal/g BW), weight, and adiposity for the animals subjected to SG with or without omentectomy was observed, and a significant reduction in adiposity in the STD group compared with the HFD group was found.

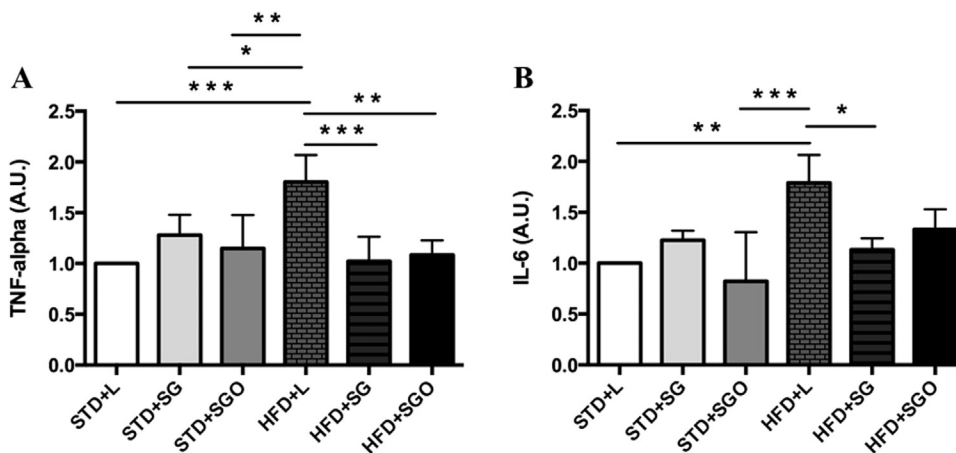


Fig. 4. Analysis of mRNA expression of inflammatory-related target genes by quantitative real time-polymerase chain reaction in the periepididymal adipose tissue of rats fed a standard diet (STD) or high-fat diet (HFD) and subjected to sham surgery (STD+L or HFD+L), sleeve gastrectomy (STD+SG or HFD+SG), or sleeve gastrectomy + omentectomy (STD+SGO or HFD+SGO). (A) mRNA expression of tumor necrosis factor-alpha (TNF- $\alpha$ ) (Arbitrary Unit). (B) mRNA expression of interleukin-6 (IL-6) (Arbitrary Unit). \* $P < .05$ ; \*\* $P < .01$ ; \*\*\* $P < .001$  (1-way ANOVA and Bonferroni posttest).

A previous study has shown that the weight loss after the SG procedure occurs even when animals are maintained on an HFD after surgery, which agreed with the results of this study [19]. In our study, the inclusion of omentectomy did not result in a statistically significant increase in weight loss compared with the group subjected to only SG. Regarding the glycemic profile, both the STD and HFD groups found a significant reduction in the levels of insulin and C-peptide after SG or SGO. Moreover, the HFD group found a significant decline in the glycemic levels, but the decline was similar in the groups with or without omentectomy. In the HFD group, the SG and SGO procedures both significantly reduced the total cholesterol levels to a similar extent, but this effect was not found for the levels of HDL (no difference between groups) and triglycerides (significant reduction in the STD+SG group only).

Only a few studies have been published that discuss the effects of omentectomy associated with bariatric surgery on the metabolic profiles and anthropometric data. Thus, this subject remains controversial and challenging. Thorne et al. reported the long-term beneficial effects of omentectomy associated with adjustable gastric banding on the weight loss and improvement of metabolic parameters in obese patients [5]. Csendes et al. presented a prospective randomized study involving 70 patients with obesity degree III in which the performance of gastric bypass with or without omentectomy was evaluated during a 2-year follow-up period [20]. During this period, no differences regarding the body mass index and plasma levels of glucose, insulin, total cholesterol, and triglycerides were found between the groups with or without omentectomy [20].

Wu et al. performed laparoscopic SG with or without omentectomy in 40 obese patients [21]. In the omentectomy group, a partial enterectomy was also performed with a resection ranging from 80 to 200 cm of the small bowel with the preservation of the first 100 cm of the jejunum and the last 200 cm of the ileum. Even in this complex technique, the 12-month follow-up found that there were no significant differences in the body mass index loss or in the percentage of excess weight loss, and the follow-up also indicated that there was no significant difference concerning the levels of triglycerides, total cholesterol, HDL, fasting glucose, and fasting insulin. Thus, in the follow-up period of this previous study, the SG procedure with or without omentectomy and partial enterectomy did not have significant effects on weight loss. The authors in this study considered that the SG procedure alone is an effective and well-tolerated procedure, and they highlighted that the inclusion of omentectomy and partial enterectomy may present a higher risk for complications, such as leakage, stenosis, and bowel adhesion [21].

However, Santoro et al. described an association among SG, omentectomy, and jejunectomy, which was named “digestive adaptation,” with favorable results regarding the improvement of co-morbidities, especially T2D [22].

These authors attributed these results to a neuroendocrine postprandial increment as indicated by the reduction of ghrelin and resistin as well as an increase of glucagon-like peptide-1 and peptide YY without the deleterious effects of malabsorption [22]. These same authors also found the beneficial effects of the entero-omentectomy alone on the glycemic profile of patients with T2D and obesity degree I during a 3-year follow-up period [23]. Heap et al. also presented favorable results regarding the improvement of co-morbidities associated to obesity when performing a technique similar to the Santoro group, which included a subtotal lateral gastrectomy, placement of a gastric ring, partial resection of the jejunum, and omentectomy [24]. However, as this technique had 4 components, it is difficult to define the real beneficial role of an omentectomy.

In our study, an interesting result was the significant weight increase of the interscapular brown adipose tissue (g/BW) observed in the HFD group subjected to SG with omentectomy. Bucerius et al. evaluated the bariatric surgery effect on the metabolic activity of different adipose tissues, and they found an increment of brown adipose tissue with an improvement of the metabolic parameters in morbidly obese patients [25]. Schneck et al. found that a decrease in white adipose tissue and an increment in brown adipose tissue occurred after SG in HFD-induced obese mice [26]. However, to the best of our knowledge, there are no studies correlating the potential effects of omentectomy associated with bariatric surgery in brown adipose tissue.

Adipose tissue is recognized as an endocrine organ because it expresses and secretes several factors with important endocrine functions [1]. The expression of proinflammatory cytokines, including TNF- $\alpha$ , has been reported in adipose tissues of different rodent models of obesity and diabetes, and these cytokines have been shown to have an important role in insulin resistance [27]. Xia et al. performed total endoscopic resection of the omentum in rats, and they found a decrease in the levels of TNF- $\alpha$  with an improvement in metabolic patterns [28]. In the present study, we observed a decrease in TNF- $\alpha$  expression in periepididymal adipose tissue in the HFD groups subjected to SG compared with the control group (sham surgery), but no significant difference was found between the groups with or without omentectomy.

In the same tissue, a significant reduction in IL-6 expression was found only in the HFD group subjected to the SG procedure. Herrera et al. performed a randomized study with 22 patients who were subjected to a laparoscopic Roux-en-Y gastric bypass with or without omentectomy. After a postoperative follow-up period of 12 months, comparative results were found in the patients with or without omentectomy regarding the glycemic profiles, lipid profiles, and the inflammatory mediators. The surgical time was significantly more prolonged when an omentectomy was performed and a duodenal perforation occurred during this procedure [29]. Sdralis et al. also reached similar

conclusions when performing SG alone or with omentectomy in 31 patients with Class III obesity [30]. The 1-year follow-up period found a comparable weight loss in both groups, an increase in adiponectin levels, an increase in HDL, as well as a significant decrease in the levels of insulin, IL-6, and high-sensitivity C-reactive protein. However, no significant differences between the groups were found, and no significant modification was found in the levels of TNF- $\alpha$  [30].

## Conclusion

A limitation in our study was the short follow-up period devoted to the animals (only 4 wk), which prevented a better assessment of the weight regain after the SG procedure. Nevertheless, other authors have found the maintenance of weight loss in the same rodent models using the same surgical technique during a 15-week post-operative follow-up period [18]. In accordance with previous studies, our study concluded that omentectomy associated with SG compared with SG alone does not result in significant benefits in the glycemic profiles, lipid profiles, and inflammatory marker expression (TNF- $\alpha$  and IL-6) in white adipose tissue of rats fed a standard and HFD. However, in the HFD group in which the omentectomy procedure was performed, there was a significant increase of brown adipose tissue. As the omentectomy procedure requires more surgical time, it involves the potential risks for other intraoperative complications. Due to the resection of a structure with proven benefits, more studies are required to obtain consistent data proving that the reduction of visceral adipose tissue with the resection of the great omentum associated with bariatric surgery improves the metabolic profile of obese patients while reducing the cardiovascular risk and other co-morbidities associated with obesity.

## Disclosure

*The authors report no proprietary or commercial interest in any product mentioned or concept discussed in this article.*

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Thaísa Soares Crespo and Antônio Sérgio Barcala Jorge contributed to the design of the study as well as the acquisition, analysis and interpretation of data. Thaísa Soares Crespo and João Marcus Oliveira Andrade helped to draft and revise the manuscript, and Alfredo Maurício Batista de Paula, André Luiz Sena Guimarães and Sérgio Henrique Sousa Santos contributed to the final approval of the version to be submitted.

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