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RESEARCH ARTICLE

Effects of Sleeve Gastrectomy on the Metabolic Profile and on the Expression of Renin-Angiotensin System in Adipose Tissue of Obese Rats

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Abstract: ***Background*:** Sleeve gastrectomy (SG) has been used as a multipurpose surgical procedure for the treatment of obesity.

***Objectives*:** The present study aimed to assess the effects of SG on the metabolic and inflammatory profile and renin-angiotensin system (RAS) expression in the white adipose tissue of male rats with obesity induced by a high-fat diet.

***Methods*:** Male Wistar rats were treated with a standard diet or high-fat diet and submitted to SG or sham surgery. The glycemic and lipid profiles and gene expression of inflammatory markers and RAS components in adipose tissue were evaluated.

***Results*:** SG led to weight loss, decreased adiposity ($p < 0.01$) and a reduction in plasma glucose ($p < 0.05$), C-peptide ($p < 0.05$), insulin ($p < 0.001$) and total cholesterol ($p < 0.05$) levels. In addition, SG led to a decrease in the expression of tumor necrosis factor-alpha (TNF-α) ($p < 0.01$), interleukin-6 (IL-6) ($p < 0.001$), angiotensinogen (AGT) ($p < 0.001$) and angiotensin converting enzyme (ACE) ($p < 0.05$) and increased the expression of angiotensin converting enzyme 2 (ACE2) ($p < 0.05$) in white adipose tissue. No statistically significant differences were observed for AT1 ($p = 0.10$) and Mas ($p = 0.22$) receptors.

***Conclusion*:** This study showed that SG leads to weight loss and improves metabolic parameters. Changes in the expression of RAS components and of inflammatory molecules in adipose tissue seem to play a role the before beneficial effects of the SG.

Keywords: Obesity, bariatric surgery, inflammation, renin-angiotensin system, metabolism, sleeve gastrectomy.

1. INTRODUCTION

Obesity is considered to be a serious public health problem worldwide. In adipose tissue, especially visceral tissue, there is an increase in the proinflammatory cytokines expression leading to the development of a chronic inflammatory state [1]. The comprehension of preventive and therapeutic strategies for the obesity remains inconclusive [2].

In this perspective, the renin-angiotensin system (RAS) may be considered a potential target for the treatment of obesity due to its metabolic properties recently described, especially in the adipose tissue [3-5].

In the last few years, several studies showed the RAS role on the metabolic function of many organs and tissues [6, 7]. It has been suggested that Ang-(1–7) suppresses the production of reactive oxygen species via attenuation of the NAPDH oxidase activity, and consequently beneficially affecting diabetic nephropathy db/db mice [8]. Additionally, Liu and colleagues. demonstrated that Ang-(1-7) exerts a protective role against oxidative stress and improves glucose metabolism in adipose tissue cells via increase of adiponectin levels and decrease in the mRNA expression of NAPDH oxidase [9]. Another study, published by Macedo et al. shows that the treatment with the ACE2 activator (diminazene aceturate – DIZE) modulates the expression of the

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renin-angiotensin markers, AGT, ACE and ACE2 in the white adipose tissue, by decreasing the adipogenesis related genes expression, such as ACC and FAS. Moreover, Wistar rats treated with enalapril, an ACE inhibitor, showed an improvement in the metabolic parameters and increase in the life expectancy [10]. In the liver, studies have shown that the renin-angiotensin system also plays an important role in the progression of chronic liver diseases. In this context, ACEi and ARBs are potential therapeutic alternatives for the treatment of liver diseases [11]. The Ang-(1-7) is shown to improve the liver inflammation through the downregulation of IL-6 and TNF-α by inhibiting resistin [12].

In addition, another clinical characteristic associated with cardiovascular impairments is the insulin resistance. This relationship was studied in clinical trials and experimental studies, showing that an improvement in hyperglycemia was observed where the RAS was inhibited by the use of either ACE inhibitors or AT1 antagonists [13, 14].

Along with clinical approaches, surgical techniques that were originally developed for the treatment of severe obesity constitute an important therapeutic approach for the treatment of diabetes mellitus type II (DM2) and other comorbidities in obese patients [15, 16]. Among those techniques, the sleeve gastrectomy (SG), which was initially indicated as the first stage in the treatment of patients with severe obesity or high surgical risk, has been considered to be a therapeutic option as a primary and definitive procedure in the treatment of obesity and DM2 due to its satisfactory results on the glycemic and lipid profiles [17, 18].

In this perspective, this study aims to evaluate the effects of the SG on the metabolic profile and tissue expression of inflammatory and renin-angiotensin system markers of rats with diet-induced obesity.

2. MATERIALS AND METHODS

2.1. Animals and Experimental Diets

The experiment was conducted with forty male Wistar rats (eight weeks old), which were randomly divided into four groups (n=10/each) and fed with the respective experimental diets for eight weeks: a standard diet (STD) or a high-fat diet (HFD). The STD (Purina - Labina®, USA), which is used for the regular maintenance of the rats, is composed of 50.30% of carbohydrate, 41.90% of protein and 7.80% of fat, with a total of 2.18 kcal per 1 g of diet [12, 19]. The HFD is composed of 24.55% of carbohydrate, 14.47% of protein and 60.98% of fat, representing a total of 5.28 kcal per 1 g of diet [12, 19]. All of the high-fat diet components were purchased from Rhoster® LTDA (São Paulo, Brazil). After this period, the animals were submitted to surgical treatment: sham surgery (laparotomy) or sleeve gastrectomy (SG). The experimental groups were: rats treated with a standard diet and submitted only to laparotomy (STD+L), rats with a standard diet and sleeve gastrectomy (STD+SG), rats with a high-fat diet and laparotomy (HFD+L) and rats with a high-fat diet and sleeve gastrectomy (HFD+SG). After surgery, the animals continued to receive their respective diets for four additional weeks. The animals were individually housed in an environment with a 12 hour light cycle (7 am to 7 pm), at a temperature of 22 ± 2.0 °C and had access to food and water ad libitum. This study was approved by the Ethics Committee of Experimentation and Animal Welfare of Unimontes, Montes Claros, Brazil, by the process number 031/2014.

2.2. Surgical Procedures

The animals were anesthetized with ketamine (100 mg/kg) and xylazine (30 mg/kg) intraperitoneally under spontaneous ventilation. Antibiotic prophylaxis was performed by intramuscular administration of ceftriaxone (100 mg/kg), immediately after the onset of anesthesia. Median longitudinal incision was performed, starting at the epigastrium, with approximately 5.0 cm in length. The groups STD+L and HFD+L underwent a sham surgery. The groups STD+SG and HFD+SG underwent SG with resection of 80% of the stomach, including the complete removal of the gastric fundus and confection of a gastric tube from the distal part of the antrum (1.5 mm from the pylorus) to the Hiss angle using 5-0 polygalactin yarn.

2.3. Measurements of Body Weight, Food Intake, Tissue Collection and Plasma Parameters

Body weight (BW), food intake and energy intake (food intake in kcal) were measured three times a week both in the pre and postoperative periods. One week before surgery and sacrifice, insulin sensitivity tests (IST) were performed for the determination of blood glucose levels, which were monitored at 0, 15, 30, and 60 min after intraperitoneal injection of insulin (0.75 U/kg BW; Sigma, St. Louis, MO, USA). The glucose tolerance test (GTT) was performed measuring the blood glucose levels at 0, 15, 30, 60, and 120 minutes after intraperitoneal injection of glucose (2 g /kg BW) following a fasting period of 12 hours, using Accu-Check (Roche Diagnostics Corp. Indianapolis, USA) [20]. Overnight-fasted rats were sacrificed by decapitation with a guillotine, and blood samples were collected and centrifuged (3200 rpm for 10 min) when the plasma was separated for the determination of the total serum cholesterol, HDL cholesterol, triglycerides, glucose, insulin, C-peptide, ferritin and C-reactive protein (CRP) levels by enzymatic tests (DSA BioELISA, USA). Samples of adipose tissues were collected, weighted and immediately frozen in liquid nitrogen and stored in dry ice (-80 °C) for further analysis.

2.4. Reverse Transcription and Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR)

Total RNA from the adipose tissue was prepared using TRIzol reagent (Invitrogen Corp., San Diego, CA, USA), treated with DNAse and reverse transcribed with MMLV (Invitrogen Corp.) using random hexamer primers. Levels of the interested genes were determined by qRT-PCR using the SYBR Green reagent (Applied Biosystems, Grand Island, NY, USA) in a QuantStudio™ 6 Flex Real-Time PCR System equipment (Applied Biosystems, USA). Gene expression was normalized to the endogenous Beta-actin (FW: 5'-TGA CAG GAT ACA GAA GGA GA-3'; RV: 5'-TAG AGC CAC CAA TCC ACA CA-3'). The genes of interest and respective primers were: TNF-α (FW: 5'-ATG GGC TCC CTC TCA TCA GT-3'; RV: 5'-GCT TGG TGG TTT GCT ACG AC-3'), IL-6 (FW: 5'-GTC AAC TCC ATC TGC CCT TCA-3'; RV: 5'-GAA GGC AAC TGG CTG

GAA GT-3'), AGT (FW: 5'-CCT AAC TGA CCC GAG CTG TAG-3'; RV: 5'-TGT GGA CTT GCT TCT GTG TGT-3'), ACE (FW: 5'-ATT GCA GCC GGG CAA CTT-3; RV: 5'-TCC TCC GTG ATG TTG GTG TC-3'), ACE2 (FW: 5'-GCC CAA AAG ATG AAC GAG GC-3'; RV: 5'-CGC TTG ATG GTC GCA TTC TG-3'), MAS receptor (FW: 5'-GCC ATG AAT ACC TCC AGC AG-3'; RV: 5'-GCT CAT GAT GAC CCA GTG C-3') and AT1 receptor (FW: 5'-CCA TTG TCC ACC CGA TGA AG-3'; RV: 5'-TGC AGG TGA CTT TGG CCA C-3').

2.5. Hematoxylin and Eosin Staining

Periepididymal adipose tissue samples were fixed in a formaldehyde solution (10%), and embedded in paraffin. Sections of 5 mm were prepared for hematoxylin and eosin (HE) staining, and were evaluated under a conventional light microscope (Olympus BX50 microscope (Tokyo, Japan). Images of fat tissue areas (X40 objective lenses) were captured with an Evolution LC color light camera (Media Cybernetics, Rockville, MD, USA).

2.6. Statistical Analysis

All data were transferred to GraphPad Prism software (Version 5.0®, San Diego, CA, USA) and analyzed with 95% ($P < 0.05$) confidence. Data are expressed as the means ± SD. The statistical significance of the differences in the mean values among rats groups was assessed by one-way ANOVA followed by the Bonferroni post-test.

3. RESULTS

During the preoperative period, the food intake (g/g BW) was similar between the groups treated with STD or HFD, but the energy intake (kcal/g BW) was higher for the HFD treated group, which led to the statistically significant increase in the body weight of the rats in this group (Figure **1**). After surgery, a significant reduction of the food intake, energy intake and body weight was observed in the groups submitted to STD+SG and HFD+SG. In these groups, a significant adiposity reduction in the white adipose tissues (periepididymal and retroperitoneal) related to the adipocyte area and diameter was observed (Figure **2**).

For the glycemic profile, a significant increase in the insulin resistance and a decrease in the glucose tolerance were observed in the HFD group pre surgery. The glycemic parameters, including measurements of IST and plasmatic measurements of glucose, C- peptide and insulin, showed a significant improvement in the post surgery period in the HFD+SG group when compared to the HFD+L and STD+L groups (Figures **3** and **4**).

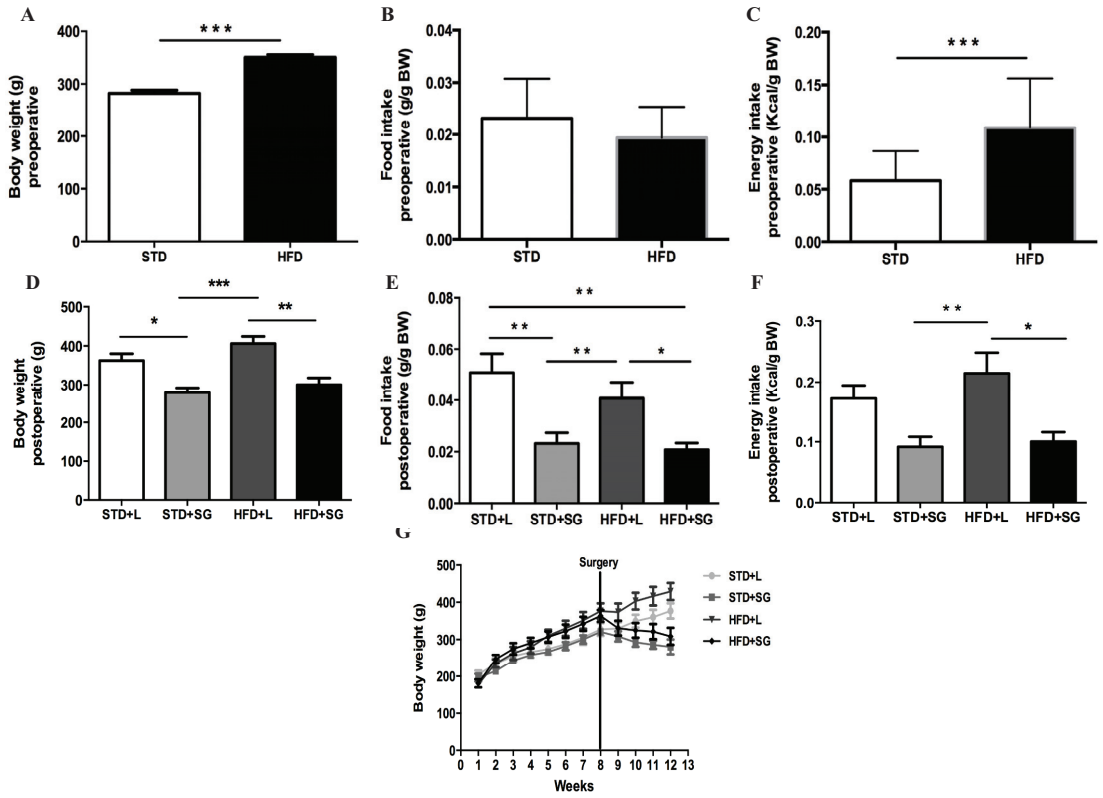

Figure 1. Body weight, food intake and energy intake of rats fed with a standard diet (STD) or a high-fat diet (HFD) and submitted to sleeve gastrectomy (STD+SG and HFD+SG) or sham surgery (STD+L and HFD+L). **(A)** Preoperative body weight (g). **(B)** Preoperative food intake (g/BW). **(C)** Preoperative energy intake (Kcal/g BW). **(D)** Postoperative body weight (g). **(E)** Postoperative food intake (g/BW). **(F)** Postoperative energy intake (Kcal/g BW). **(G)** Pre- and postoperative weight curves. *P < 0.05; **P < 0.01; ***P < 0.001 (t-tests, one-way ANOVA and Bonferroni post-test).

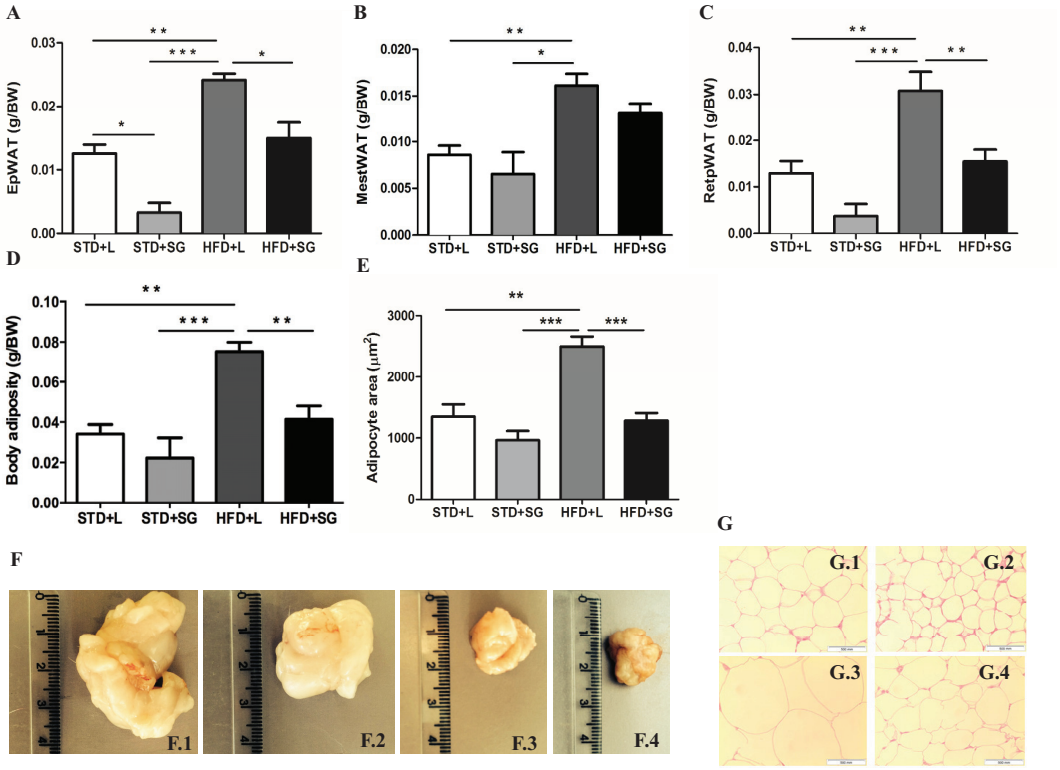

Figure 2. Effects on the adipose tissues of rats fed with a standard diet (STD) or a high-fat diet (HFD) and submitted to sleeve gastrectomy (STD+SG and HFD+SG) or sham surgery (STD+L and HFD+L). **(A)** Periepididymal adipose tissue weight (g/BW). **(B)** Mesenteric adipose tissue weight (g/BW). **(C)** Retroperitoneal adipose tissue weight (g/BW). **(D)** Body adiposity/white adipose tissue weight (periepididymal, mesenteric and retroperitoneal) (g/BW). **(E)** Adipocyte area (µ/m²). **(F)** Periepididymal adipose tissues. **F.1**: HFD+L; **F.2**: STD+L, **F.3**: HFD+SG; **F.4**: STD+SG **(G)** Hematoxylin and eosin (HE) staining of periepididymal adipose tissue samples. Inlet: HE staining. Scale bar: x40. **G.1**: STD+L; **G.2**: STD+SG; **G.3**: HFD+L; **G.4**: HFD+SG. *P < 0.05; **P < 0.01; ***P < 0.001 (one-way ANOVA and Bonferroni post-test).

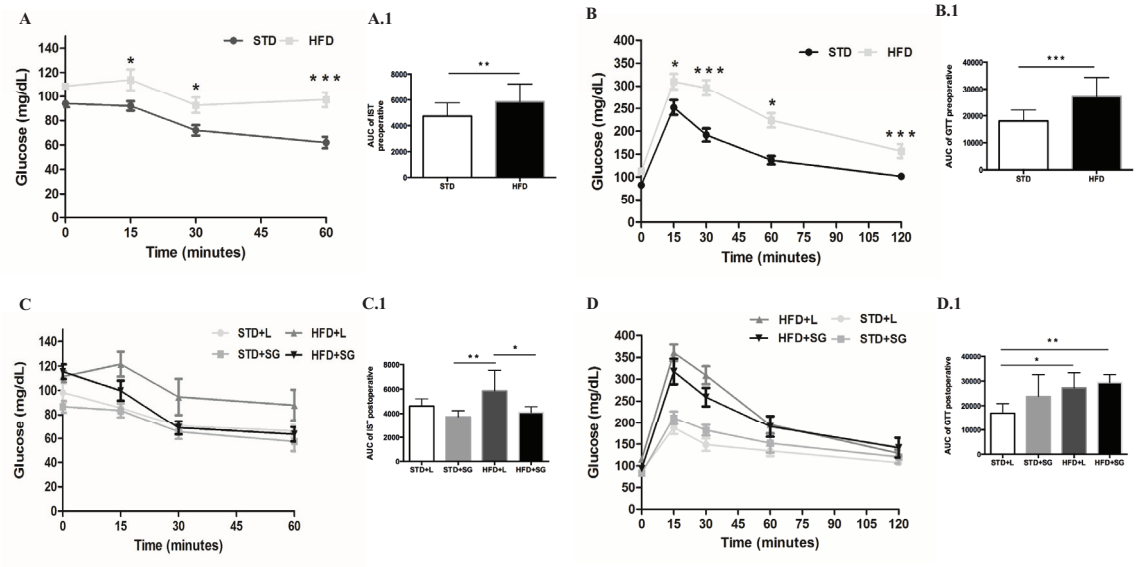

Figure 3. Preoperative and pre-sacrifice insulin sensitivity tests (IST), glucose tolerance tests (GTT) and their respective areas under the curve (AUC) in rats fed with a standard diet (STD) or a high-fat diet (HFD) and submitted to sleeve gastrectomy (STD+SG and HFD+SG) or sham surgery (STD+L and HFD+L). (**A**) Preoperative IST (mg/dL). A.1: AUC of preoperative IST. (**B**) Preoperative GTT (mg/dL). B.1: AUC of the preoperative GTT. (**C**) Pre-sacrifice IST (mg/dL). C.1: AUC of the pre-sacrifice IST. (**D**) Pre-sacrifice GTT (mg/dL). D.1: AUC of the pre-sacrifice GTT. *P < 0.05; **P < 0.01; ***P < 0.001 (one-way ANOVA and Bonferroni post-test).

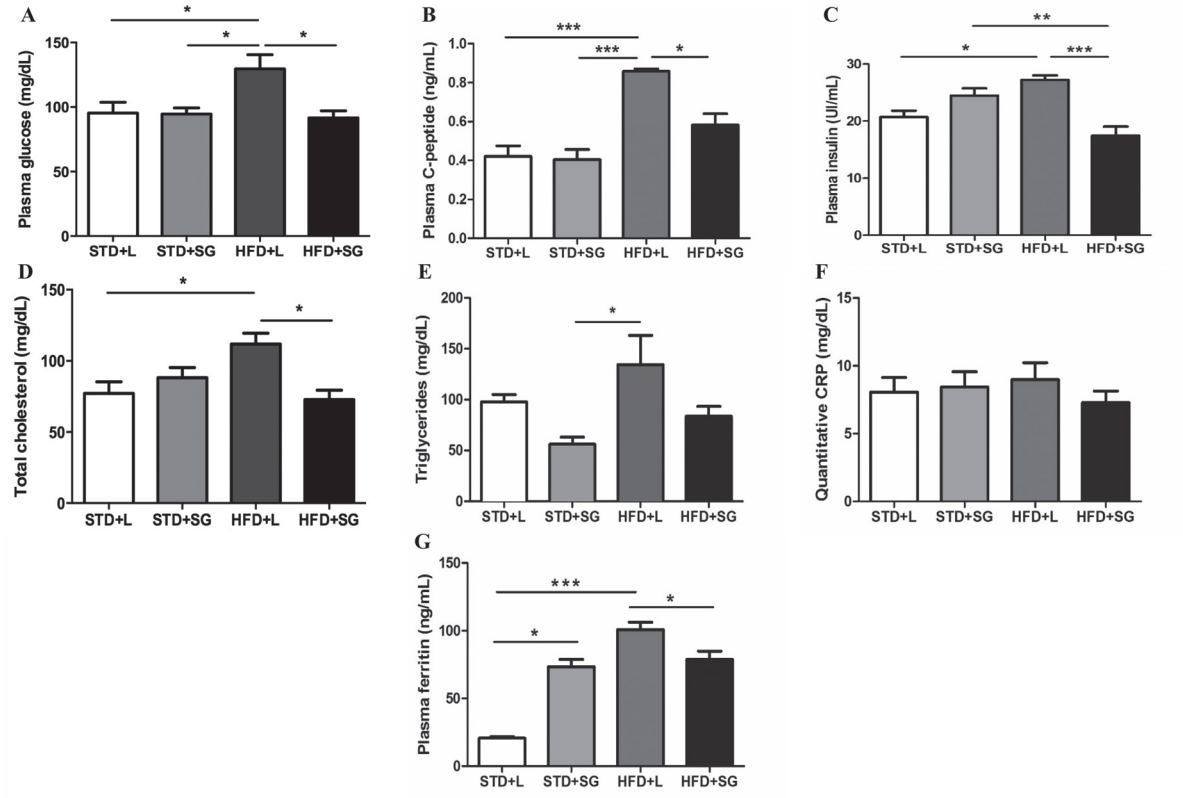

Figure 4. The blood parameters of rats fed with a standard diet (STD) or a high-fat diet (HFD) and submitted to sleeve gastrectomy (STD+SG and HFD+SG) or sham surgery (STD+L and HFD+L). (**A**) Plasma glucose (mg/dL) (**B**) Plasma C-peptide (ng/mL) (**C**) Plasma insulin (UI/mL) (**D**) Plasma total cholesterol (mg/dL) (**E**) Plasma triglycerides (mg/dL) (**F**) Plasma quantitative CRP (mg/mL) (**G**) Plasma ferritin (ng/mL). *P < 0.05; **P < 0.01; ***P < 0.001 (one-way ANOVA and Bonferroni post-test).

For the lipid profile, a decrease in the levels of total cholesterol was observed in the HFD+SG group in comparison to the HFD+L and STD+L groups. Additionally, a decrease in the levels of triglycerides was observed in the group STD+SG in comparison to the HFD+L group. Regarding the HDL cholesterol levels, there was no difference between the groups submitted to bariatric surgery when compared to the respective control groups, which was also observed for the quantitative plasmatic measurements of CRP (Figure **4**). The ferritin levels were significantly lower in the group HFD+SG in comparison to the respective control group.

The mRNA expression of proinflammatory cytokines by qRT-PCR in the periepididymal adipose tissue showed a statistically significant decrease in the levels of TNF-α and IL-6 in rats from the HFD+SG group in comparison to its respective sham group (Figure **5**). The expression of components of the RAS by qRT-PCR in the same tissue showed a decrease in the mRNA levels of angiotensinogen and ACE and an increase in the mRNA levels of ACE2 (Figure 5). No statistically significant differences were observed for the AT1 and Mas receptors.

4. DISCUSSION

In the present study, we evaluated the effects of SG on the glycemic, lipid and inflammatory profiles as well as on the expression of renin-angiotensin system components in the white adipose tissue of rats with obesity induced by a high-fat diet.

As far as we know, this study is unique regarding the analyses of gene expression of the RAS in the periepididymal adipose tissue after SG, evidencing a significant decrease in the levels of AGT and ACE and an increase in the levels of ACE2, although no differences were observed for the AT1 and Mas receptors among groups. Previous studies demonstrate that the adipose tissue represents an important source of the RAS's components production and that there is an interaction between the secretions of the RAS's tissue components with the RAS's circulating components. The hypertrophy of the adipocytes increases the secretion of RAS's components, enabling the generation of several cardiovascular and metabolic changes [21, 22]. At the cellular level, RAS's components induce insulin resistance, and contribute to the oxidative stress, inflammation and apoptosis in β-pancreatic cells [23]. On the other hand, experimental study showed the importance of the Ang-(1-7) on the lipid and glycemic profiles as well as on the inflammatory profile in the adipose tissue. High chronic levels of Ang-(1-7) are shown to attenuate the proinflammatory profile of the adipose tissue, protecting against metabolic stress induced by a high-fat diet [24]. The increase in the plasma levels of Ang-(1-7) are also described to generate an increment in the glycemic and lipid profiles, as evidenced by the improvement in

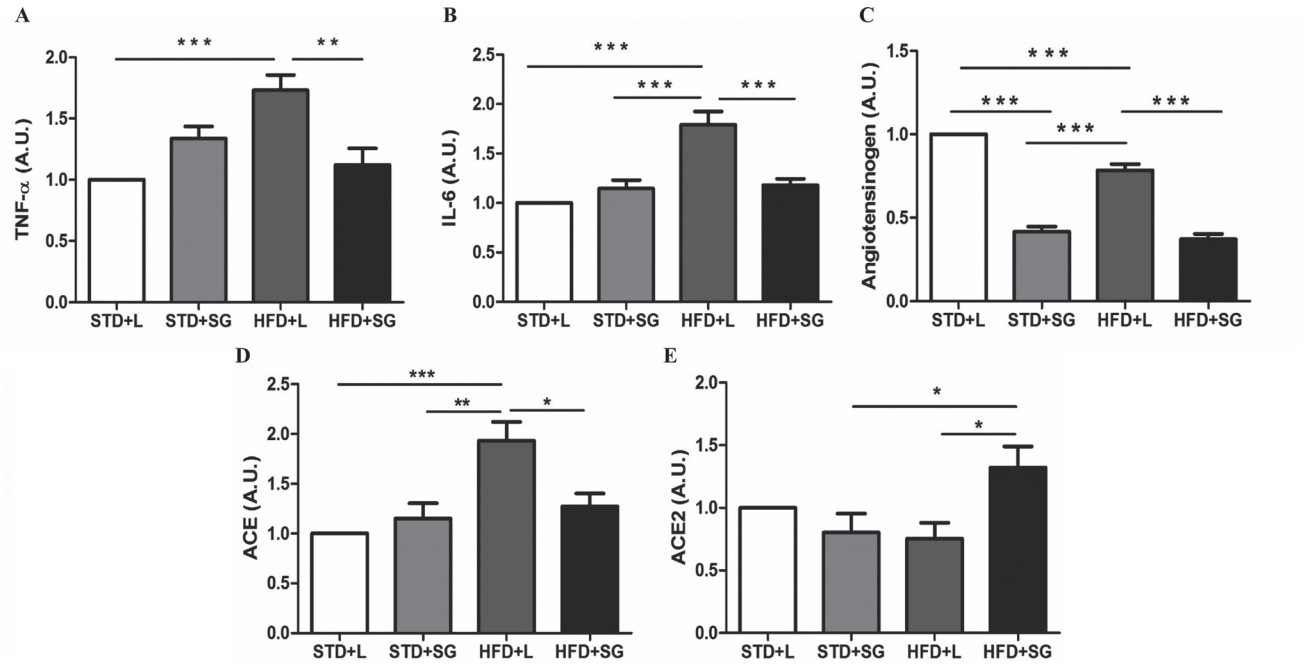

Figure 5. Expression of the inflammatory-related targets and components of the renin-angiotensin system by qRT-PCR in the periepididymal adipose tissue of rats fed with a standard diet (STD) or a high-fat diet (HFD) and submitted to sleeve gastrectomy (STD+SG and HFD+SG) or sham surgery (STD+L and HFD+L). (**A**) mRNA expression of tumor necrosis factor - alpha (TNF- α) (Arbitrary Unit). (**B**) mRNA expression of interleukin-6 (IL-6) (Arbitrary Unit). (**C**) mRNA expression of angiotensinogen (AGT) (Arbitrary Unit). (**D**) mRNA expression of angiotensin-converting enzyme (ACE). (**E**) mRNA expression of angiotensin-converting enzyme II (ACE2) (Arbitrary Unit). *P < 0.05; **P < 0.01; ***P < 0.001 (one-way ANOVA and Bonferroni post-test).

the glucose tolerance and insulin sensitivity, as well as a decrease in cholesterol and triglycerides levels [20].

There is evidence in the literature that this experimental model is suitable for the induction of obesity, the accomplishment of SG and the follow-up of body profile (weight loss and adiposity) and biochemical and hormonal parameters [25, 26]. The SG performed in this study showed a reduction in food intake and a significant weight loss in the postoperative period. This data is in accordance with the literature that demonstrates a weight loss in humans and rodents after SG [25, 27].

The decrease observed in the food intake and food consumption of the rats submitted to SG, might be associated to several mechanisms. The renin-angiotensin system seems to have an important role in the regulation of the food intake and body weight. Corroborating, Santos and colleagues already showed that the treatment with enalapril reduced body fat, food intake and energy intake. These findings may be associated with decreased leptin levels [10].

In this study, the GTT and IST performed in the preoperative period exhibited altered glycemic profiles induced by a high-fat diet. In the postoperative period, the glucose plasma levels, C-peptide and insulin confirmed the improvement in the glycemic profile in the groups treated with a high-fat diet and submitted to SG. This effect is widely documented in the literature, where the authors have shown the surgical effect on the glycemic profile, with a significant reduction in the glucose plasma levels and insulin and a normalization of the homeostatic model assessment for insulin resistance [28].

The renin-angiotensin system acts directly in the glycemic homeostasis. ACEi demonstrated important effects in the improvement of insulin resistance and glucose tolerance through the increase in the glucose uptake in the adipose tissue, skeletal muscle and liver. Especially in the skeletal muscle, the ACEi administration increases the GLUT-4 translocation, thus favoring the glucose uptake by this tissue. The ACE2 deficiency, in contrast, worsened the insulin resistance and glucose intolerance induced by diet [29]. Additionally, increased Ang-(1-7) circulating levels resulted from chronic infusion or transgenic expression, had beneficial effects on hyperinsulinemia, insulin resistance and inflammation in the adipose tissue of rats [30], and increased insulin sensitivity and glucose tolerance in norm-glycemic rats [20]. Moreover, chronic oral treatment with an Ang-(1-7) formulation had a hypoglycemic effect in a type 2 diabetes rat model, where the insulin sensitivity was improved [31].

Regarding the lipid profile, a statistically significant reduction in the total cholesterol levels was demonstrated, corroborating with studies that showed an improvement in the lipid profile after SG in both rodents and humans [32, 33], although the same was not observed with the dosage of HDL cholesterol and triglycerides levels.

The lipid profile seems to be directly modulated by the RAS. Santos and colleagues showed that in TGR(A1-7)3292 animals with increased Ang-(1-7) plasma levels, decreased

levels of triglycerides and cholesterol levels are observed [20]. Moreover, Feltenberger and colleagues [7] demonstrated that mice treated with Ang-(1-7) presented decreased plasma levels of total cholesterol and triglycerides as compared to the control group. Furthermore, in this study, a decrease in the SREBP-1c expression levels, a cholesterol and fatty acids synthesis activator in the liver, was found. Thus, Ang-(1-7) seems to have an important effect on *de novo* lipogenesis and lipid metabolism in the liver, consequently improving the lipid parameters [7].

Concerning the inflammatory parameters, in our study the CRP plasma dosage did not show statistically significantly difference among the groups. Obesity evolves with increasing CRP plasma concentrations, being considered to be an important risk factor for the development of cardiovascular diseases. A study performed in patients with severe obesity evidenced high concentrations of hs-CRP, which were significantly reduced after SG [34]. However, the ferritin dosage showed a statistically significantly reduction in the high-fat diet group submitted to SG. This result is in accordance with a study that showed ferrigin decreased levels in obese patients one year after SG [35]. The inflammatory markers are increased in obesity and have been described to be a link between obesity and its comorbidities, mainly due to their proinflammatory properties and consequent alterations in the cardiovascular function and insulin resistance state [36]. In this study, TNF-α and IL-6 were decreased in the periepididymal adipose tissue of rats submitted to SG, corroborating with a study that demonstrated the beneficial effects of this surgical technique in the reduction of the inflammatory state associated with obesity [36].

Furthermore, the RAS modulates the expression of several inflammatory markers in the white adipose tissue. Some results have shown the role of the Ang-(1-7) in the downregulation of inflammation and the following changes associated, in the white adipose tissue, liver and kidney [4, 37]. Recent studies have shown that Ang-(1-7) is able to decrease the proinflammatory markers expression in rodents white adipose tissue [4] and to ameliorate the nephropathy in diabetic Zucker rats [37]. Additionally, two reports indicated the role of the RAS in the modulation of the hepatic inflammatory process. Santos and colleagues demonstrated that Ang-(1-7) ameliorates the liver inflammatory state through a decrease in the expression of IL-6 and TNF-α by inhibiting the resistin expression [12]. In addition, Feltenberger *et al*. indicated that Ang-(1-7) reduced ACC, PPAR-γ and SREBP-1c levels in the liver, thus suggesting the inhibition of the lipogenesis as the cause of the amelioration of steatosis and decreased expression of IL-6 and TNF-α expression.

In summary, the primary goal of the surgical treatment of obesity, in addition to weight loss, is the improvement of the metabolic profile. This study, showed for the first time the effects of SG on the white adipose tissue RAS expression (reduced levels of AGT and ACE ad increased levels of ACE2), which acts on the glycemic, lipid and inflammatory profiles in rats with obesity induced by diet. Giving the experimental characteristic of our study, further studies are necessary to understand the described effects on the human obesity. The assessment of Ang II and Ang-(1-7) tissue levels and the serum levels of the renin-angiotensin system markers was not performed and may comprise the main limitations of our study.

CONCLUSION

Finally, in this experimental study, the sleeve gastrectomy leads to weight loss and improves metabolic parameters. Changes in the expression of RAS components (AGT, ACE and ACE2) and inflammatory molecules in the adipose tissue seem to play a role on the sleeve gastrectomy beneficial effects gastrectomy. Altogether, these findings open our perspectives to the comprehension of the SG role in the body and metabolic homeostasis.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

This study was approved by the Ethics Commit-tee of Experimentation and Animal Welfare of Unimontes, Montes Claros, Brazil, by the process number 031/2014.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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