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Development and testing of an accessible and reproducible experimental model of sleeve gastrectomy in rats

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Abstract:

Based on the results of bariatric surgery on metabolic syndrome (MS) that are not exclusively associated with weight loss, it is pertinent to analyze the modifications of MS blood variables, inflammatory biomarkers, and oxidative stress in an experimental model. The experimental models for this purpose described so far are complex, expensive, and difficult to reproduce.

An experimental analytical study was carried out using male Wistar rats. The animals were divided into 6 groups of 12 rats each: control (C), metabolic injury (MS), sleeve gastrectomy (SG), MS + SG for 6 (SG6), 12 (SG12), and 24 (SG24) weeks. Oxidative and metabolic variables, weight loss, and food intake were evaluated. MS was induced by administration of 10% fructose diluted in drinking water for 6 weeks.

Of the initial 72 rats, 4 (5%) died after surgery. The oxidative and metabolic variables showed significant differences when comparing the MS group with the rest of the groups with a progressive improvement in the groups operated on for SG.

The model proved to be safe and reproducible. The metabolic injury generated and the results obtained from SG tended to emulate those observed in humans.

Keywords:

- Experimental Surgery
- Bariatric Surgery
- Oxidative Stress
- Sleeve Gastrectomy
- Wistar Rats

Introduction

Knowledge of the pathophysiology of obesity and the beneficial mechanisms that bariatric surgery triggers are needed to allow the development of effective therapies. In this context, animal models could be an essential tool to understand the regulation of body weight and variations in metabolic pathways after bariatric interventions1. Despite this, protocols based on animal models have not been standardized and are often difficult to reproduce, leading to contradictory results[2, 3].

The objective of this report is to propose a simplified and easily reproducible animal model of metabolic injury and sleeve gastrectomy and to analyze the behavior of the oxidative and metabolic parameters to verify the effect of the applied interventions.

Material and Methods

An experimental analytical study was carried out using male Wistar rats that were kept in the animal room of the Faculty of Medical Sciences of the National University of Córdoba in Córdoba, Argentina. The rules for handling and housing the animals followed the CICUAL guidelines (Institutional Committee for the Management and Care of Laboratory Animals) (n. 49/17).

A total of 72 rats divided into 6 groups were analyzed. Metabolic involvement was induced by the administration of 10% fructose diluted in drinking water for 60 days.

Each group consisted of 12 animals that were studied sequentially in different experimental situations:

I) Control (n = 12). "C"

II) Water diet supplemented with 10% fructose for 60 days (n = 12). "MS"

III) Sleeve gastrectomy (n = 12). "SG"

IV) Water diet supplemented with 10% fructose in water for 60 days. + Sleeve gastrectomy and 6 weeks after surgery (n = 12). "SG6"

V) Water diet supplemented with 10% fructose in water for 60 days. + Sleeve gastrectomy and 12 weeks after surgery (n = 12). "SG12"

VI) Water diet supplemented with 10% fructose in water for 60 days. + Sleeve gastrectomy and 24 weeks after surgery (n = 12). "SG24"

No preoperative fasting was performed. The selected rats were anesthetized with intraperitoneal 0.8 ml ketamine by a puncture at the left Mc Burney point. A 1 cm incision was made in the midline. The stomach was pulled from the greater omentum into the incision and extracted through the incision. Vascular clamps were used, and the stomach was sectioned, thus avoiding contamination and bleeding. A suture was made with 5-0 polipropilene with a small round needle and the clamp was released. Then a new suture was performed ensuring a gastric restriction of at least 80%. Hemostasis was controlled and the stomach was reintroduced into the cavity. The abdominal wall was closed in one plane and finished with cutaneous synthesis. The rats remained in a warm environment until they woke up under strict observation. They started with water enriched with fructose 10% ad libitum in the immediate postoperative period. Ad libitum diet was restarted. (Video 1)

Blood was obtained by puncture of the cava vein from animals previously anesthetized with ketamine (0.8ml intraperitoneal) (Video 2). Plasma fibrinogen, nitric oxide (NO), superoxide dismutase (SOD), myeloperoxidase (MPO), fasting blood glucose, lipid profile (TG, LDL, HDL, total cholesterol), and TG / HDL-c index were measured.

Bodyweight was observed and recorded every day for the first 2 weeks after the operations and then once every 2 weeks until death.

To analyze the results of the continuous variables, multiple comparisons were used by Student's "t" test. A significance level of $p < 0.05$ was established for all cases.

Results

The procedure time from anesthesia was 48 ± 13 minutes. Of the initial 72 rats, 4 (5%) died after surgery. In all cases, death occurred within the first three days. Necropsies were performed on all dead animals: 2 died from bleeding, 1 from fistula, and in 1 case there were no alterations in the abdomen, and it was assumed that oral intolerance and dehydration were the cause of death.

The results of the oxidative plasma variables: fibrinogen, NO, SOD and MPO are summarized in figure 1. The MS group showed deteriorated values that reached statistical differences in all the variables analyzed. On the other hand, the groups operated for SG showed a progressive improvement in the values analyzed until reaching statistically significant differences when compared with MS and losing statistical differences when compared with C.

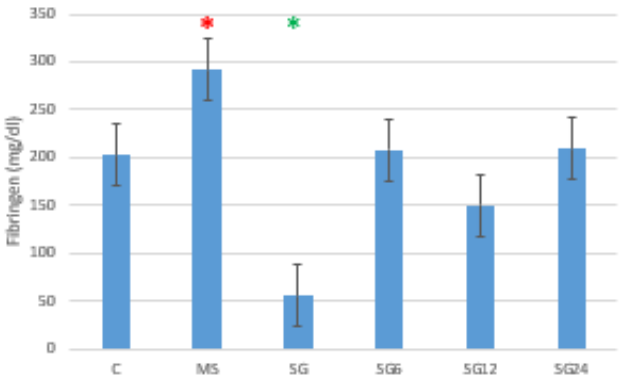

Fig. 1.a: Fibrinogen values in all groups. *MS vs C, SG, SG6, SG12, SG24 p<0.001. *SG vs C, SG6, SG12, SG24 p<0.001.

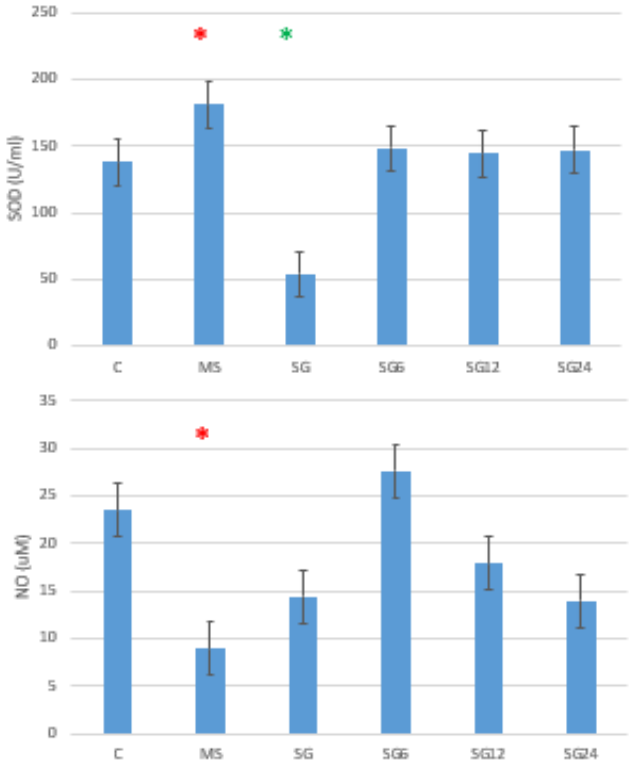

Fig. 1.b: Nitric Oxide values in all groups. *MS vs C, SG, SG6, SG12, SG24 p<0.001.

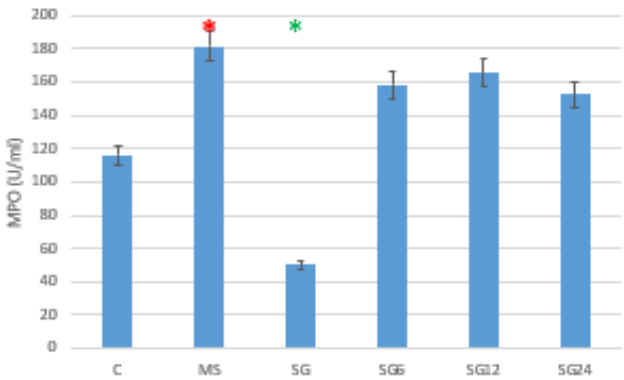

Figure 1. Evolution of oxidate plasmatic variables: a. Fibrinogen, b. Nitric Oxide, c. Superoxide Dismutase, d. Myeloperoxidase

Glycemia was maximum in the MS group (235 ± 10.7) and showed statistical differences concerning the rest of the groups (p <0.001 in all cases). No differences were found when comparing C (126 ± 2.4) with SG (116.25 ± 19), SG12 (147.71 ± 12), and SG24 (131.83 ± 10.4) or these groups with each other. SG6 (174.6 ± 14.2) showed significantly higher glucose levels than C, SG12, and SG24 (Fig. 5). The same pattern was observed when studying the behavior of triglycerides, total cholesterol, LDL, and HDL.

Finally, the TG/ HDL index was compared between groups. The MS group index (6.2 ± 1.8) was the highest and statistical differences were reported when compared with the rest of the groups (p <0.001 in all cases). (Fig. 2)

No differences in body weight were observed when comparing the groups on the first day of the experience. At the time of surgery, after 6 weeks of fructose 10% in drinking water, the MS, SG6, SG12, and SG24 groups showed significant weight gain compared to C and SG (p = 0.001). No differences were obtained when comparing C with SG or when comparing MS, SG6, SG12, and SG24 with each other. At this point, the MS group was sacrificed for metabolic evaluation.

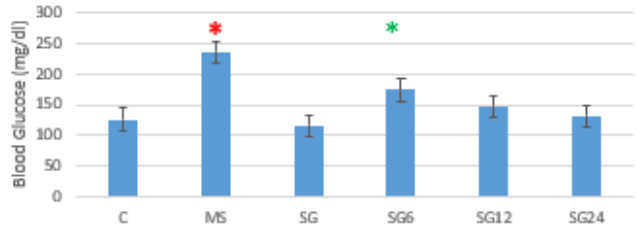

Fig. 2.a: Blood glucose values in all groups. *MS vs C, SG, SG6, SG12, SG24 p<0.001. *SG6 vs C, SG12, SG24 p<0.001.

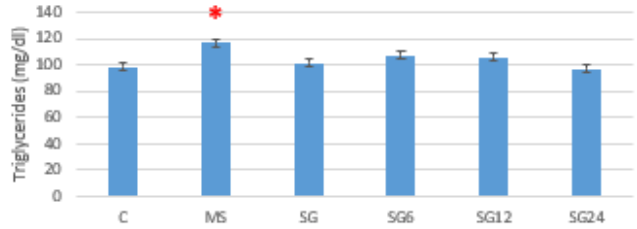

Fig. 2.b: Triglycerides values in all groups. *MS vs C, SG, SG6, SG12, SG24 p<0.03.

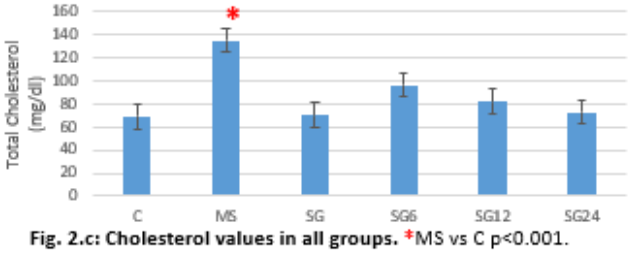

Fig. 2.c: Cholesterol values in all groups. *MS vs C p<0.001.

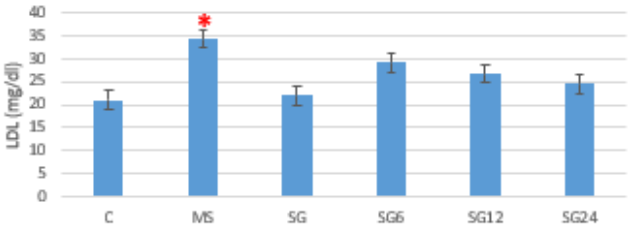

Fig. 2.d: LDL values in all groups. *MS vs C p<0.001.

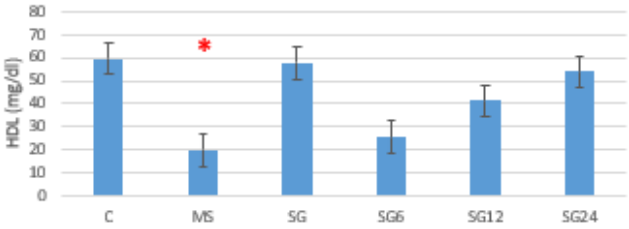

Fig. 2.e: HDL values in all groups. *MS vs C p<0.001.

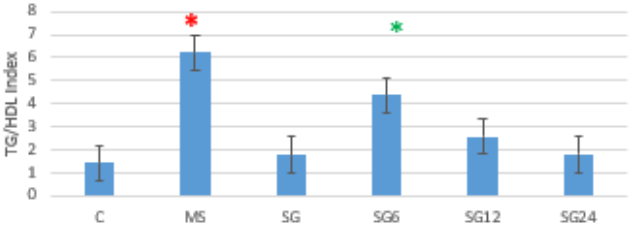

Fig. 2.f: TG/HDL values in all groups. *MS vs C, SG, SG6, SG12, SG24 p<0.001. *SG6 vs C, SG12, SG24 p<0.001.

Figure 2. Evolution of metabolic variables: a.Blood glucose, b. Triglycerides, c. Total Choresterol, d. LDL, e. HDL, f.TG/HDL

Discussion

The use of an experimental model using fructose-fed rats has shown to develop a metabolic injury in animals whose biochemical profile shows alterations comparable to the human condition4. It should be noted that a significant elevation of the TG / HDL index is associated with low insulin sensitivity and metabolic syndrome5. After SG, the differences tended to decrease towards values more like the control group. This could correspond to a metabolic improvement that other authors have also reported. It should be mentioned that this variable could highlight the reproducibility of the model since the measurement of insulin in rodents is complex, not very accessible, and expensive since the laboratory test to be applied is species-specific and increases costs and availability.

In recent years it has been shown that subclinical chronic inflammation could be a relevant pathophysiological factor in the development of insulin resistance and consequently MS6. In our experience, the decrease in NO entails the presence of oxidative stress since NO indirectly quantifies the concentration of reactive oxygen species, especially 02-.

In an oxidative environment, NO combines with the excess of 02- producing ONOO- which is a powerful free oxygen radical7. Finally, the activity of fibrinogen, MPO, and SOD increased in the MS group. These variables are currently considered as oxidative biomarkers for different pathological processes that lead to an important inflammatory state8.

Different experimental models were proposed for the study of SG and its impact on obesity and metabolic syndrome in rats. The novelty of our model is the inclusion of an accelerated recovery protocol in animals. In our design, preoperative fasting was abolished and both liquids and solids were provided after ad livitum surgery. This decreased the handling of the animals to provide subcutaneous hydration in the immediate postoperative period. On the other hand, neither cannibalism nor self-injurious behaviors feared by many authors were observed. This could be due to the minimal incision made which in turn reduces the chances of peritoneal contamination. The anesthesia was performed with ketamine, which is a cheap, accessible, easy to use, and very safe drug since it does not cause respiratory depression and does not need special equipment to be administered. The animals wake up quickly with good analgesia, an issue that also responds to the small size of the incision. We use a continuous 5-0 suture in two planes, but we consider that any material could be used, although mechanical sutures increase costs (Table 1). The sham surgeries and the pairfed groups have not shown unexpected results in any of the experiences published in the literature, so we decided not to use them to avoid unnecessary animal sacrifices, reduce costs and operating times, optimizing logistics.

AUTHOR	ANIMAL (N)	FOLLOW UP	DEATHS	DIET	FASTING PREOP	ANES-THESIA	CLOSURE	DIETA LÍQUIDA	LIQUIDS INTAKE	INTAKE AFTER SG	WEIGHT	META-BOLIC ASSES-MENT
CASTELAN 2007 (9)	Wistar (8)	7 weeks	1*	Standar feed	12 hs	keta-mine and xylaci-na IP	6-0 pro-pylene	After surgery	72 hs	-	Decrea-se	-
LÓPEZ 2009 (10)	ZF (12)	14 days	No	Standar feed	4-6 hs	Iso-fluora-ne	3-0 silk	After surgery	48 hs	Decrease	Decrea-se	Improve-ment
CHAM-BERS 2010 (3) PRE-TRA-TAMIENTO (KG/M2)	Long-Evans (52)	125 days	-	High fat	24 hs	Iso-fluora-ne	Stapler	After surgery	72 hs	Decrease	Decrea-se	Improve-ment
BRUINSMA 2015 (1)	Spra-gue-Dawley (10)	8 weeks	No	High fat	Overni-ght	Iso-fluora-ne	Stapler	24 hs	Day 7	Decrease	Decrea-se	Improve-ment
BRINC-KERHO FF 2011 (11)	Spra-gue-Dawley (18)	4 weeks	5 + 3 excluded	High fat	7 days	Iso-fluora-ne	Stapler + 5-0 PDS + omentum	After surgery	Day 7	-	Decrea-se	Improve-ment
PEREFERER 2008 (12)	Spra-gue-Dawley/ ZF/ZDF	2 weeks	Non	High fat + High Kcal	8 hs	Tileta-mine + Zolaze-pam IM	5-0 popylene	After surgery	72 hs	Decrease (specially S-D)	De-crease (specia-lly S-D)	Impro-vement (specially S-D)
SIGNORINI 2021	Wistar (72)	6-24 weeks	4	Stan-dard feed + fructo-se	NO	keta-mine IP	5-0 vycril	After surgery	After surgery	Decrease	Decrea-se	Improve-ment

Table 1. Summary of the most influencing SG experimental model reported.
**Informed a pilot trial with 8 deats out of 9 sugested to be due to non-invaginating suturing and early fed*

Conclusions

The proposed sleeve gastrectomy model has shown to be safe regarding animal survival as well as obtaining the desired effects after the induction of metabolic injury and sleeve gastrectomy. This model could provide a reproducible and accessible option for any group seeking to develop research protocols on the matter.luego de la inducción de la afectación metabólica y la realización de la gastrectomía en manga. Este modelo podría aportar entonces una opción reproducible y accesible para cualquier grupo que busque desarrollar protocolos de investigación en la materia.

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