Metabolic and Weight Changes After Bariatric Surgery in a Rat Model of Induced Type 2 Diabetes Mellitus and Obesity

AMADEUS DOBRESCU, LAURIAN STOICA, MIHAI MITULETU, ALEXANDRU ISAIC, CIPRIAN DUTA, GABRIEL VERDES, CRISTI TARTA*, ALINA HEGHES, LAZAR FULGER

University of Medicine and Pharmacy Victor Babes Timisoara, 2 Eftimie Murgu Sq., 300041, Romania

Sleeve gastrectomy (SG) and gastric plication (GP) are surgical procedures providing weight loss through several mechanisms incompletely understood. Rat models allow the possibility of tight regulation of experimental conditions, making them the ideal candidates for animal models in bariatric surgery. The aim of this study was to compare the weight and metabolic changes between sleeve gastrectomy and gastric plication in a rat model with type 2 induced diabetes mellitus and obesity. Fifteen male Wistar rats were fed with DIO food (Bio Serv ®F3282 - Mouse Diet, High Fat, Fat Calories -60%), after 36 weeks were allocated to the study three arms-SG, GP and sham operation (SO). Four weeks after the surgery the rats were weighted again. Blood tests were performed before surgery and four weeks after surgery searching for blood glucose, total cholesterol, HDL, triglycerides, and LDL. At the onset of the study, the rats were 9 weeks old and had an average body mass of 231.6g ± 31.58. After 36 weeks of DIO, one day preoperatively body weight was 774.93g ± 95.02. The preoperatively average of body weight in the SG group was 777.4g ±104.66, 775.4 g ±104.6 in the GP group, respectively 772g ±79 in the SO group. 4 weeks after surgery the mean body weight in the SG group was 648.8g ±99.09, in the GP group was 695.6g ±99.09, respectively 825.4g ±79.87 in the SO fed ad libitum group. There was a significant decrease of mean fasting glucose levels at 4 weeks postoperative in the SG group compared to the SO group (87.4mg/dL±8.73 versus 103.6 mg/dlL±4.66, p= 0.01). The same trend of mean fasting glucose was registered in the GP group versus the SO group (92.8 ± 5.67 mg/dl vs 103.6 mg/dl ± 4.66, p=0.01). Our study provides evidence of the positive effects of bariatric surgery for treating patients with morbid obesity associated with diabetes mellitus and dyslipidemia and the use of rats to study the mechanisms of weight reduction and metabolic changes in bariatric surgery.

Keywords: sleeve gastrectomy, gastric plication, metabolic changes, dyslipidemia

Obesity and diabetes mellitus (DM), as vital components of the metabolic syndrome, have steadily increased over the past years [1]. Current trends favor a sedentary lifestyle with minimal physical activity and high-energy intake, leading to insulin resistance. These two components are not independent diseases but rather interact as both causes and effects [2]. Out of the 422 million people suffering from diabetes mellitus, 90% have type 2 DM (T2DM), with overweight being the main contributing factor [1]. Lipid profile disorders are common in obese patients with T2DM. Given that obesity is associated with many adverse effects on cardiovascular structure, function, and hemodynamics, it constitutes a major risk factor for several cardiovascular diseases such as hypertension, coronary heart disease or stroke [3]. Thus, weight loss represents an improvement for various risk factors as well as numerous cardiovascular pathologies associated to obesity [4].

Since traditional methods such as diet, physical activity, and drugs have failed to attain long-term therapeutic effect on obesity, diabetes, and dyslipidemia, new strategies have been developed, which include bariatric surgery. Currently, restrictive bariatric surgery procedures including adjustable gastric banding, sleeve gastrectomy (SG), gastric plication (GP), gastric balloon and gastric by-pass are used in metabolic surgery. Sleeve gastrectomy is characterized by a reduction of the gastric volume through the resection of the stomach along the greater gastric curvature and construction of a tubular gastric pouch [5]. Gastric plication on the other hand can be performed through invagination of the greater gastric curvature [6].

As opposed to human studies, rat models allow the possibility of tight regulation of experimental conditions, making them the ideal candidates for animal models in bariatric surgery [7]. The results can be easily extrapolated for humans.

An extensive search of the literature has rendered relatively few results concerning gastric plication and its implications in rats.

Therefore, the aim of this study was to compare the weight and metabolic changes between sleeve gastrectomy and gastric plication in a rat model with type 2 induced diabetes mellitus and obesity.

Experimental part

Material and methods

Experimental animals. Fifteen male Wistar rats, obtained from the biobase of Pius Brinzeu Center for Flap Surgery and Microsurgery of the Victor Babes University of Medicine and Pharmacy Timisoara, were maintained in individual cages under controlled temperature (21–23 °C), humidity (50-55%), and light (12 hours light, 12 h dark, lights turned on at 7 o'clock). Only healthy animals were used in this process. All experiments were conducted having the approval of the local Ethics Committee of the Victor Babes University of Medicine and Pharmacy in compliance with the European Union laws on animal protection (86/609/EC).

Study design. At the onset of the study, the rats were 9 weeks old and were acclimatized to the facilities for one month before study commencement. During the first

* email: cristitarta@yahoo.com; Phone: (+40)726437643

phase of the experiment, the rats had free access to DIO food (Bio Serv ®F3282 - Mouse Diet, High Fat, Fat Calories -60%) and tap water during the first 36 weeks. After that, the Wistar rats were randomized into three weight-matched groups: group SG to undergo sleeve gastrectomy (n=5), group GP to undergo gastric plication (n=5) and control group SO to undergo sham surgery (n=5). After the surgical procedures, normal rat chow diet (Bio Serv® F4031) was fed to the rats until the end of experiment. Additional blood was drawn after these 4 weeks.

Surgical procedures and anesthesia protocol. After a 14 hours overnight fast with access only to water, the rats were introduced into a 20/10 cm induction chamber for anesthesia, using Isoflurane® (Anesteran 99.9% - Rompharm™) 5% in a mixture with oxygen at flow of 2.5 L/min debit based on spontaneous respiration. Once induction was achieved, isoflurane 1.5 -2.2% in mixture with oxigen was administered through a cone mask for rodents at a flow of 2-2.2L/min. All surgeries were performed under sterile conditions. The rat was shaved and povidone-iodine solution was applied to the skin.

In order to perform the sleeve gastrectomy, a 4 cm midline incision was made, the gastrosplenic ligament was divided and the stomach was externalized. Using a 10 Ch orogastric tube, the stomach was calibrated for all procedures. A vascular clamp was placed along the greater curvature from the antrum to the fundus across the stomach which helped remove, using a scalpel, the main part of the corpus and fundus of the stomach. The stomach was then closed with 5-0 non-absorbable polypropylene monofilament suture (Premilene B|Braun) in two layers in a continuous fashion (fig 1).

For the gastric plication a 4 cm midline incision was made, the gastrosplenic ligament was divided and the stomach was externalized. The gastric plication was created by imbrication of the greater gastric curvature over an orogastric tube applying a first row of extramucosal interrupted stitches of 5-0 non-absorbable polypropylene monofilament suture (Premilene B|Braun). The second row consisted of running suture lines of 5-0 non-absorbable polypropylene monofilament suture (Premilene B|Braun) (fig 2).

Fig. 1 Sleeve gastrectomy

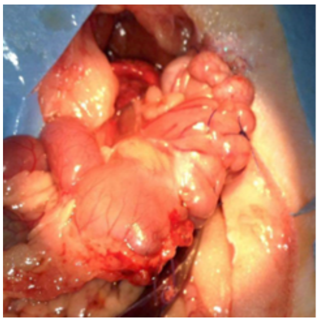

Fig. 2 Gastric plication

For the sham-operated groups, after performing a 4 cm upper midline incision, the stomach was externalized, manipulated, and then returned to the abdomen. The peritoneal cavity was cleaned with saline solution and the abdominal wall was closed with running 3-0 polyglactin acid sutures. The skin was then sewed with 3-0 running intracutaneous absorbable multifilament coated polyglactin suture (Vicryl Ethicon).

All animals were given 5 mL sterile warmed saline intraabdominally to avoid dehydration and allowed to recover spontaneously from anesthesia and surgery.

Postoperative Care. Prophylactic antibiotherapy consisting of ceftriaxone (10 mg/kg/daily) was administered subcutaneously immediately after surgery and for the next three days. Also, anti-inflammatories (meloxicam 1mg/kg/daily) were administered subcutaneously concomitantly. During this period the rats were allowed access to a solution of Glucose 10%. After 48 hours from the surgical procedure, all rats were fed ad libitum, with normal chow diet (Bio Serv® F4031 NJ, USA). All animals included in the study survived for the entire duration of the experiment.

Blood collection and analysis. Fasting blood glucose was measured from the tail of the rat, using an ACCU- CHEK ™ glucometer. After the laparotomy was performed, blood was prelevated from the inferior vena cava for determination of the plasma concentration of total cholesterol, triglycerides, LDL-cholesterol and HDL-cholesterol, which were measured using spectro-photometry.

A calibrated Sartorius scale was used to weight the rats. Percentage total weight loss (%TWL) was calculated as the percent of body weight loss, which was calculated from the equation: (pre-interventional body weight final body weight) × 100/pre-interventional body weight [8].

Statistical analysis. Results are shown as means ± SD. The data were collected using an experimental database called ColoRo, for an easy and facilitated track and storage of the information and a faster access. This database application was written in a modern programming language (PHP, MySql, HTML5, Javascript). ColoRo was hosted on an Apache server which enabled online access through mobile and desktop devices, most of the time smartphones were used, allowing a fast update of the variables. Dynamic sheets were created for every moment in the timeline when variables were measured. A study number identified every study subject. Data were exported to Microsoft Excel ™ for final analysis. One-way analysis of variance ANOVA and student t-test were used for comparison of the means between the groups. A p value <0.05 was considered statistically significant.

Results and discussions

At the onset of the study, the rats were 9 weeks old and had an average body mass of 231.6g ± 31.58. There was no significant statistical difference in the initial body mass of the rats among groups ($p=0.78$). After 36 weeks of DIO, one day preoperatively body weight was 774.93g ± 95.02. The preoperatively average of body weight in the SG group was 777.4g ±104.66, 775.4 g ±104.6 in the GP group, respectively 772g ±79 in the SO group. No statistical difference was registered between the groups before surgery ($p=0.99$). Figure 3 shows weight evolution before and after surgery in the three groups.

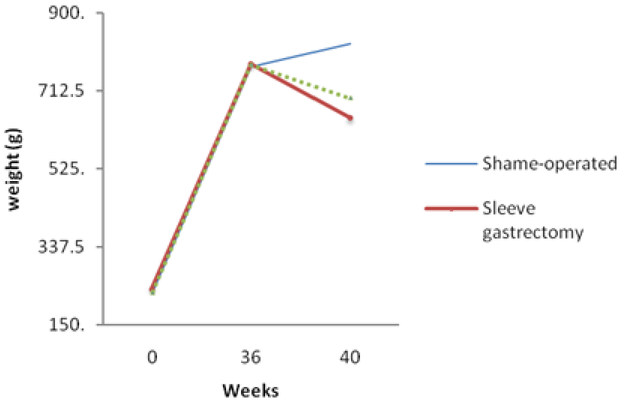

Fig 3. Weight evolution in the SG group, GP group and SO group

Four weeks after surgery the mean body weight in the SG group was 648.8g ±99.09, in the GP group was 695.6g ±99.09, respectively 825.4g ±79.87 in the SO fed ad libitum group. Comparing the SG group with the GP group at one month postoperatively there was no statistical difference regarding body weight between groups (648.8g vs 695.6 g, p>005). Regarding average body weight in the SG group versus the SO fed ad libitum group there was a significant decrease in body weight in the SG group versus the SO group (648.8g vs 825g, p= 0.01). Comparing rats submitted to GP with rats submitted to SO fed ad libitum, weight statistically decreased in the GP group (p=0.04).

There was no statistically difference between %TWL in the SG group (16.2 %) and the GP group (10.2 %) (p > 0.05) (fig 4).

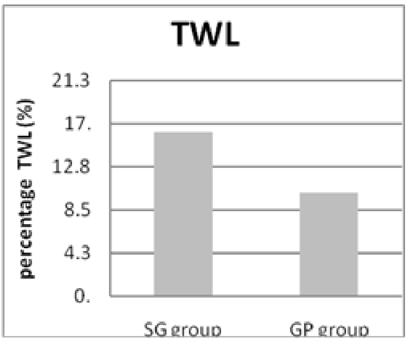

Fig 4.Total weight loss in SG group and GP group (in %)

Preoperatively, the mean fasting glucose levels for all the groups was 142.4 mg/dL ±23.78. There is no statistical significance among the three groups (p= 0.99). There was a significant decrease of mean fasting glucose levels at 4 weeks postoperative in the SG group compared to the SO group (87.4mg/dL±8.73 vs 103.6 mg/dL ±4.66, p= 0.01). The same trend of mean fasting glucose was registered in the GP group versus the SO group (92.8 ± 5.67 mg/dL vs 103.6 mg/dL ± 4.66, p=0.01). There was no difference regarding mean glucose levels between the SG group compared to the GP group (p>0.05).

There was no statistical significance between average postoperative levels of triglycerides in the SG group as compared to the GP group (150.6 mg/dL versus 153.2mg/dL, p= 0.88) (table 1). Regarding postoperative total cholesterol levels, both groups had a decreasing trend without statistical significance (p =0.68). Comparing means of the serum HDL levels in the SG group versus GP group, there was an ascending trend, without statistical significance (p=0.96). No statistical difference was registered between the SG groups compared to the GP (0.95), regarding serum LDL levels.

Obesity and its complications, such as type 2 diabetes mellitus and dyslipidemia, is one of the most challenging public health issues, representing a major medical problem, and is associated with social and economical implication, thus bariatric surgery have proven to be the most efficient long-term therapy [9-11]. Sleeve gastrectomy was initially proposed as a first step operation at high risk obese patients, but can been indicated as effective as a single procedure in patients with lower BMI [12-14]. Gastric plication was proposed as an alternative bariatric option for treating morbidly obese patients [15].

The intake of high caloric food both in humans and rodents has a vital importance in the development and maintenance of obesity [16,17]. Animal models that can mimic human physiological responses are a useful tool in investigating metabolic disease [18].

This study analyzed the weight and glycemic changes after sleeve gastrectomy or gastric plication in an obese rat animal model. Changes in the lipid profile were also evaluated. At 36 weeks, rats were divided in three weight-matched groups: undergoing sleeve gastrectomy, gastric plication and sham-operated group. There was no statistical difference among mean rat weight groups at the beginning of the surgical procedures. However, four weeks after surgery, the positive effects of sleeve gastrectomy and gastric plication were observed, the SG group registered the most important weight decrease, followed by the GP group. There was no statistical difference between the mean body weight of the SG group versus the GP group (p>0.05), however, both groups registered weight decrease. *Valentí* et al [10] showed the positive effects of sleeve gastrectomy on weight reduction in rats. Guimaraes et al [12] reported a significant decrease in body weight by performing gastric plication. At the end of experiment, the SO group registered a slow increase in weight, compared to the rapidly ascending trend registered before the surgical procedure. There was a statistically

Table 1
MODIFICATION OF LIPID PROFILE (MEANS ± SD)

	SG group			GP group			SO group		
	preop	Postop	p value	preop	postop	p value	preop	postop	p value
TG (mg/dL)	161.4 ±35.89	150.6 ±40.36	0.004	159.6 ±34.83	153.2 ±37.46	0.009	158.6 ±29.44	155.2 ±38.15	NS
TC (mg/dL)	138.4 ±14.87	127.2 ±13.27	0.006	134.6±14 .01	127.4 ±11.63	0.03	131.4 ± 13.84	129.4 ±13.81	NS
HDL (mg/dL)	52.2 ±9.09	59 ±6.35	0.008	54.8 ±9.47	59.2 ±7.94	0.01	55.8 ±9.47	56.8 ±7.52	NS
LDL (mg/dL)	95.4 ±15.56	86.8 ±14.65	0.004	94 ±13.44	87.4 ±16.41	0.04	91.6 ±12.4	90. 6 ±14..43	NS

significant difference between the weight in the SG group and the weight in the SO group (p= 0.01) as well as between the GP group and the SO group (p=0.04). On the other hand, when comparing percentage of total weight loss between the SG and the GP groups, the SG group achieved a higher %TWL then the GP group (16.2% vs 10.2), but we were unable to find any statistically significant differences (p>005). Moncada et al [8] obtained 22% percentage total weight loss after SG in rats.

The results of our study showed amelioration in fasting glucose levels in both groups (GP, SG). Rats submitted to sleeve gastrectomy showed a significant improvement of glycemic control in comparison to the shame-operated group (p= 0.01). The same trend was observed in rats submitted to gastric plication (p=0.01). Similar results have been observed by Eickhof [19] where the glycemic control was improved after sleeve gastrectomy in rats. Guimaraes et al [12] also presented an amelioration of the glycemic profile in rats, after gastric plication. Both the SG and the GP groups had decreasing postoperative fasting glucose level, there were no differences between the two techniques.

Regarding the lipid profile our results showed a significant improvement of the triglycerides levels, which based on current evidence play an important role in the process of dyslipidemic atherogenesis. By lowering triglyceride levels there is also a substantial reduction in the number of occurring CVD events. Both procedures, sleeve gastrectomy and gastric plication, ameliorate triglyceride levels, with a slight improvement noted in sleeve gastrectomy. Total cholesterol decreased for both SG and GP groups and we have noticed a limited increase in HDL levels for both groups. Moreover, LDL levels decreased significantly between the sleeve gastrectomy and the gastric plication groups. Results from the literature concerning the lipid profile vary a lot.

One limitation of our study is the fact that it was technically impossible for the same surgical team to perform all surgical procedures on the rats in the same day. Thus, there was a three-day delay between group operations, which could mean a source of bias for the weight measurements. A second limitation was that for a better assessment of the glycemic profile a HOMA-IR measurement would have been necessary.

Conclusions

Sleeve gastrectomy and gastric plication have proven to be efficient methods for weight reduction and amelioration of glycemic and lipid profile in rats. Therefore, our study provides evidence of the positive effects of bariatric surgery for treating patients with morbid obesity associated with diabetes mellitus and dyslipidemia and the use of rats to study the mechanisms of weight reduction and hormonal changes in bariatric surgery.

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