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TITLE: Gastric plication and sleeve gastrectomy in an experimental model of obesity: new insights into weight loss, intake and metabolic results

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TITLE: Gastric plication and sleeve gastrectomy in an experimental model of obesity: new insights into weight loss, intake and metabolism

ABSTRACT

Aim: Laparoscopic gastric plication (LGP) is a bariatric surgical technique based on the anatomical principles of laparoscopic sleeve gastrectomy (LSG), but its effects on the metabolic profile are still uncertain. The aim of our study is to compare the changes in weight, metabolic parameters and gastric histology following intervention by gastric plication (GP) and sleeve gastrectomy (SG) in an experimental model of obesity.

Methods: To conduct the study, thirty-two eight-week-old male Sprague Dawley rats (Charles River®) were fattened by means of a cafeteria diet and randomly assigned to the following experimental groups: Group 1: GP (n = 12); Group 2: SG (n = 12); Group 3: sham (n = 8).

Results: Unlike the SG group, the GP group attained the weight of the sham group at the end of the experiment (week 16). The GP group continued to eat more cafeteria diet than the SG group. In addition, the SG group achieved better glycaemic control than the GP group. Significantly higher plasma ghrelin levels were observed at week 16 in the GP group than in the SG Group (2.29 ±0.5 vs 1.07± 0.4, $p<0.05$), which also occurred for the glucagon plasmatic levels (62.71 ± 36.2 vs 24.63 ± 9.3, $p<0.05$).

Conclusions: GP is not as effective as SG in terms of weight loss and metabolic control. The animals subjected to a GP continued to have a high appetite for the cafeteria diet unlike the animals submitted to an SG. Hormonal mechanisms possibly related to glucagon and ghrelin may be involved in this metabolic response.

Key Words: Gastric Plication; Sleeve Gastrectomy; Ghrelin; Glucagon

Introduction: Laparoscopic gastric plication (LGP) is a bariatric surgical technique based on the anatomical principles of laparoscopic sleeve gastrectomy (LSG). The LGP technique consists of reducing the gastric volume via invagination or infolding of the gastric curvature to "simulate" a gastric tube, thereby decreasing the volume of intake. However, its effects on the metabolism have not yet been demonstrated. The works published on this technique date back to the beginning of the last century, and in 1969 Kirk RM published the first comparative experimental study of the plication of the greater curvature with respect to the plication of the gastric anterior wall [1]. However, clinical implementation of LGP has not been widespread. On the other hand, LSG, which was initially considered to be a restrictive technique, after the publication of numerous studies has become consolidated as a technique with a clear metabolic component [2]. This technique has been demonstrated to produce changes in weight as well as changes in intake and comorbidities such as diabetes mellitus. It is currently the most frequently performed surgery in the world according to the last IFSO survey [3]. Due to anatomical similarities, LSG has become the benchmark for verifying the results of LGP. Although LGP may produce similar effects as LSG on weight loss, intake regulation and carbohydrate metabolism, few studies have compared these two techniques. LGP appears to be as effective as LSG in the short term in terms of %EWL and %TWL [4]. Moreover, a few studies have analysed metabolic changes following LGP. Some of these studies have reported improvements in glucose homeostasis along with decreases in glycosylated haemoglobin, insulin and ghrelin [5]. However, as studies published to date differ widely in terms of inclusion criteria and follow-up, long-term conclusions cannot be drawn [6]. Finally, since several studies have observed an inflammatory component at the level of the plicated stomach, the long-term histological changes caused by gastric plication should also be analysed [7].

The aim of our study is to compare the changes in weight, metabolic profile and gastric histology following an intervention by gastric plication (GP) and sleeve gastrectomy (SG) in an experimental model of obesity. Therefore, this study can be defined as an applied research study since its final objective is to solve practical issues and improve existing processes.

Methods: This is an experimental and comparative prospective study of two bariatric surgical techniques (GP vs SG). To conduct the study, thirty-two eight-week-old male Sprague Dawley rats

(Charles River®) were fattened by means of a cafeteria diet and randomly assigned to the following experimental groups: Group 1: GP (n = 12); Group 2: SG (n = 12); and Group 3: sham (n = 8). To avoid the unnecessary use of animals, since the experimental protocol is well standardized, the sham group was limited to a smaller number of animals (experimental principle of reduction). We used this rat model to obviate the genetic limitation to weight loss that other strains can produce. The procedures were carried out at the animal procedure room of the Faculty of Medicine and Health Sciences of the Rovira i Virgili University in Reus (Tarragona, Spain) in accordance with the conditions established in current regulations (DOGC 214 of 30/07/1997 on the protection of animals for experimentation and other scientific purposes). All procedures performed were in accordance with the experimental ethical standards of the institutional research committee (Generalitat de Catalunya-registration number 3522). The animals were kept in metal cages with 12-hour light and dark cycles, an average temperature of roughly 22ºC, and a relative humidity of 40-60%. Guidelines on food, hygiene and behavioural issues were followed in agreement with established protocols on the general care of experimental animals. To obtain an obesity model, as we did in previous works [8,9], the animals were fattened for four weeks by means of an *ad libitum* cafeteria diet (6.250 Kcal/Kg) in the following proportions: carbohydrates (45%), proteins (10%) and fats (45%). The diet consisted of cupcakes, non-roasted peanuts, high-fat cheese, bacon, energy bars, and chocolate cookies (20gr per day per animal). The Panlab® AO4 maintenance diet (3.100 Kcal/Kg) was also administered *ad libitum* in the following proportions: 15.4% protein, 2.9% fat and 60.5% carbohydrate. Two pre-surgery glycaemic control tests were performed (15 and 25 days prior to the intervention) via puncture of the tail (coccygeal vein). The first blood samples were extracted in the week before surgery via cannulation of the jugular vein. Surgery was performed once the animals had reached 12 weeks. During the first 24 hours after surgery, the rats were administered an *ad libitum* full liquid diet (2 kcal/ml) (Resource 2.0®). From the third day after surgery and until sacrifice, the animals were again administered a solid diet consisting of 20g of cafeteria diet plus 20g of maintenance feed and water *ad libitum.* Intake was checked daily by calculating the weight of non-ingested food. Intake (kCal) of standard feed and cafeteria diet were calculated as follows:

Standard feed: Amount of standard feed (g/day)×3.173 (kcal/g of feed). Cafeteria diet: Kcal/day of standard feed + Amount of cafeteria diet (g/day)×6.25 (kcal/g of cafeteria diet).

All surgical procedures and blood samples were conducted after six hours of fasting and under general anaesthesia using Zoletil® (tiletamine and zolacepam) (20 mg/kg) administered intraperitoneally. When required during surgery, anaesthesia was maintained via administration of 50% of the initial dose. The animals were sacrificed at the end of week 16 by anaesthetic overdose. A second blood sample was taken by intracardiac puncture and a sample of the plicated area of the stomach was taken for histological study. The schedule for these procedures is shown in Figure 1.

All blood and tissue samples were processed in the University's experimental surgery laboratory and stored at -80ºC (plasma) and in formaldehyde (tissues) until their final joint determination. Biochemical and histological determinations were conducted at the laboratories of the Pere Virgili Research Institute (IISPV), which is affiliated to the University. The metabolic profile (insulin, ghrelin, GLP-1, glucagon, leptin and peptide Y) was determined using ELISA techniques (Milliplex map Rat Metabolic Hormone Panel-Metabolism Multiplex Assay (RMHMAG-84K, MERCK Millipore®)).

Surgery: Sleeve gastrectomy group: A midline laparotomy 4 cm in length was performed. After the structures were identified, the greater curvature was dissected and the short and gastroepiploic arteries were ligated with 4/0 silk. The incision line for the longitudinal sleeve gastrectomy was defined using two bulldog clamps to minimize the leakage of gastric contents. Linear gastrectomy was performed with a cold scalpel and posterior double continuous suture from the fundus to the antrum with 4/0 polypropylene. When necessary and to prevent leakage, sutures were applied with loose stitches in weak areas of the gastric suture.

Gastric plication group: A midline laparotomy 4 cm in length was performed and the greater curvature was dissected as per the SG. Invagination of the gastric greater curvature was performed using polyethylene cannulas. Continuous sutures were applied via invagination in a caudocranial direction from the antrum to the rumen using 4/0 polypropylene.

Sham group: A small gastrostomy (0.5 cm) was performed and closed immediately.

Closure of the abdominal wall in all animals was performed on musculoaponeurotic tissue with 3/0 polypropylene continuous sutures and on skin with rapid absorption 3/0 polyglycolic acid continuous sutures. To facilitate postsurgical hydration, a 5 cc bolus of 5% glucosaline serum was injected subcutaneously.

The sample size was calculated using the Sample Power 2.0 software. For a minimum power of 80% and α of 0.05, we chose the value that requires the greatest "n" for significance, which in this case was weight loss. We therefore needed 12 animals for each of the main study groups (SG and GP) and 8 animals for the sham group. To compare means within the same model, we used the Student-Fisher t test. When the assumption of normality was not fulfilled, we used the non-parametric Wilcoxon W test for paired data. To compare means between two different models, we applied the Student-Fisher t test for independent samples; otherwise we used the non-parametric Mann-Whitney U test. We considered p to be significant below 0.05. To analyse our results we used the IBM-SPSS 22.0 statistical software.

Results: Weight and intake: Figure 2 A and 2B show the growth curves for the SG, GP and Sham groups in comparison with the normal growth curves for male Sprague-Dawley rats fed with normal feed. Unlike the SG group, the GP group attained the weight of the sham group in week 16. Significant differences in the increases in total weight were observed between week 13 and week 16 for all groups except the SG group ($p < 0.05$). The animals in both the GP group and the SG group significantly decreased their food intake (cafeteria diet and feed) after surgery. However, no significant differences were found in food intake after surgery for the sham group. Table 1 shows the numerical results for weight and intake. When we divided the total kilocalories ingested by each group into the intake of cafeteria diet and feed, we found significant differences in the intake of cafeteria diet between the GP group and SG group. Differences in the feed intake were also observed, though these were not significant (see Figure 3).

Glycaemia: Two weeks after surgery, there was a statistically significant decrease in the average blood glucose levels of the GP group (down to 82.58 mg/dl). However, two weeks later these levels increased once more to levels similar to those before surgery (162.92 mg/dl). Average blood glucose levels in the SG group also decreased significantly two weeks after surgery (down to 95.08 mg/dl), and continued to

decrease throughout the postsurgical period. By the end of the study the average glycaemia level for these animals was 79.5 mg/dl. The sham group, on the other hand, remained hyperglycaemic throughout the experiment. Our numerical results are shown in Table 2. Figure 4 illustrates the differences between the surgical groups. The individual results of each animal are shown in Figure 2C.

Biochemical parameters: All groups in this study had similar average plasma ghrelin levels before intervention. No significant differences between the SG and GP groups were found in the comparative analyses at week 12. However, significantly higher plasma ghrelin levels were observed at week 16 in the GP group. No significant differences between the SG and GP groups in plasma insulin levels were found in the comparative analyses at week 12 or at week 16. We found significant differences in the GLP-1 levels between the SG and GP groups at week 16. When glucagon was analysed separately for the three groups between weeks 12 and 16, we found significant differences for both the GP group and the SG group. Moreover, there were significant differences between these two groups at week 16. Finally, significant differences in PYY were found only for the SG group before and after surgery. The numerical values for metabolic markers are shown in Table 3.

Gastric biopsy sections taken 4 weeks after GP showed evidence of fibrosis and chronic inflammatory gigantocellular reaction to a foreign body in the submucosa, which was probably related to the intervention (see Figure 5A). The same inflammatory reaction was also observed in the SG group, though without associated fibrosis (see Figure 5B). Figure 5C shows the image corresponding to the sham group.

The postsurgical complications observed in this study were: three intraabdominal collections in the SG group, found by chance during sacrifice, at the level of the antrum/prepyloric zone and two eventrations after midline laparotomy (one in the GP group and one in the SG group).

Discussion: Gastric plication is not a new technique. In 1900 Mayo Robson reported a series of 28 gastric plications, concluding that as a bariatric technique it could be suitable in the absence of pyloric obstruction. The rise in bariatric surgery and its success in recent decades has led to the search for new techniques that provide the same benefits as traditional ones. Along with the success of SG, this has led to the resurgence of GP. Indeed, numerous groups have published results of applying this technique to

humans to imitate the effects of SG. The objective is to theoretically equate the results of the two techniques, thereby avoiding having to perform gastric resection and thus minimizing the undesirable complications of SG, such as leakage, dehiscence of the staple line or bleeding. However, GP is not without complications, such as vomiting, stenosis or gastroesophageal reflux [10,4]. Our results on weight showed that growth in the sham and GP groups accelerated during the postsurgical period and that the animals in the GP group weighed more than those in the SG group when sacrificed. It may be said *a priori* that for experimental animals, gastric plication is not as effective a bariatric technique as SG [11]. As several studies have shown, both techniques lead to an initial weight loss. However, bearing in mind that four weeks for rodents is approximately equivalent to two and a half years for humans [12], GP appears to be ineffective in the medium term. This is consistent with recently published data on humans in this area [4].

When we compared total intake in Kcal at week 12 with total intake at week 16, we found that both the SG group and GP group consumed fewer calories at the end of the study. This result was not observed for the sham group, which even consumed slightly more food in week 16. Both GP and SG are techniques that experimentally lead to a decrease in intake [13]. However, when we decomposed total food intake into feed and cafeteria diet consumption, we found significant differences between the two types of food. The SG group had less appetite for the cafeteria diet and consumed a greater proportion of feed, whereas the opposite was true for the GP group. This phenomenon has also been observed in other studies that analysed the effects of sleeve gastrectomy experimentally [14,15,16]. No doubt, the physiopathology behind this phenomenon should be studied in terms of the response of the central nervous system. This may well explain the before-and-after-surgery changes in glycaemia levels for both groups. Clinical studies published in this area have apparently found no differences between the two techniques when the patients are non-diabetic; however, as with our experimental results, they have found differences when the patients are diabetic [17,18].

The stomach is the main producer of ghrelin, so the differences observed between the two techniques can be justified since in SG we remove the gastric fundus (main producing area) while in GP it is not removed. Plasma ghrelin levels decreased in the SG group, though not significantly, between week 12

and week 16. We expected to observe more striking decreases that would be in agreement with published clinical studies. However, our results do agree with those of experimental studies on ghrelin and sleeve gastrectomy [19,20,21,22]. Ma GJ et al, for example, observed a 17% drop in ghrelin levels in the same weeks post-surgery, similarly to our study [23]. In other studies with a longer postsurgical follow-up, the decrease in ghrelin levels is even more evident [24,25,26,27,28,29]. Some studies have reported an initial increase in ghrelin levels four weeks after intervention followed by a decrease after 12 weeks [30.] The fact that the decrease in postsurgical ghrelin levels was only slight may be because ghrelin can be released not only by the gastric fundus but also at different levels in the organism [31,32]. In the GP group, on the other hand, there was a progressive increase in plasma ghrelin levels and the differences observed between weeks 12 and 16 were highly significant statistically. These results have also been found in several human studies that showed increases in plasma ghrelin levels after gastric plication and other techniques such as gastric band surgery [33]. Weight loss may therefore lead to an increase in ghrelin levels as a counterbalance [34,35]. Ghrelin levels in the sham group remained stable. The clinical results of GLP-1 levels found following bariatric surgery vary greatly. Fasting plasma levels may remain stable or increase but after stimulation tests with food they normally increase [36], especially after Roux-en-Y gastric bypass and, to a lesser extent, after sleeve gastrectomy [37,38]. The GP group showed a slight increase in GLP-1 after surgery, though this increase was not statistically significant. Contrary to our expectations, the SG group showed a slight decrease in GLP-1 in week 16 that was also not statistically significant. Although postsurgical GLP-1 levels were higher for the GP group, this increase did not make up for the glucose levels, which were still higher than for the SG group. Despite the higher GLP-1 levels in the GP group, GP appears to be less effective in controlling diabetes than SG. Although several studies have not observed any before-and-after-surgery differences that would justify our results [39], an overly restrictive gastric resection may have slowed down gastric emptying, thereby justifying the differences in GLP-1 levels observed between the two groups at week 16 [40]. Although the GLP-1 decreases the gastric emptying rate, gastric emptying is determined by the type of surgical technique and, in the case of SG, the size of the remaining antrum is a key point. Distances from the pylorus of 3 cm produce an increase in emptying speed greater than larger distances

[41]. Even so, a very short distance to the pylorus (<1cm) could produce the opposite effect and behave like a semioclusive pattern [42].

The PYY levels in the GP group were slightly higher at week 16 than before surgery. In the SG group PYY levels also increased and were slightly higher than those of the GP group, though these differences were not statistically significant. PYY levels in the sham group, on the other hand, remained stable. These results are in agreement with those in the literature, where most studies have also observed slight increases in PYY after SG [37,38]. Other studies, however, have not observed any changes in these levels [39].

Like insulin, glucagon is a hormone that regulates glucose homeostasis. It is secreted by the alpha cells of the pancreas in response to hypoglycaemia, promoting gluconeogenesis and glycogenolysis [43]. The interrelationship between the alpha and beta cells of the pancreas is altered in patients with type-2 diabetes mellitus (probably in relation to peripheral insulin resistance). Therefore, although diabetics show hyperinsulinaemia and hyperglycaemia, glucagon is uninhibited and remains high, thus worsening the patient's hyperglycaemia [44]. In week 16 our study reported an increase in fasting glucagon levels in the gastric plication group and a slight decrease in the sleeve gastrectomy group. These between-group differences were statistically significant. This increase in glucagon at week 16 in the GP group may explain the increase in glucose observed during that week. In this context, a recent randomized clinical study reported greater increases in glucagon after GP than after SG and RYGBP [17]. Beyond the incretin profile and insulin plasma levels, glucagon should play a greater role in the study algorithm for the metabolic response to surgery.

Surgery-linked discrete chronic inflammation and gigantocellular reaction in the submucosa were observed in stomach biopsies taken during sacrifice of the SG and sham groups. However, in the GP group, fibrosis and chronic inflammation with gigantocellular reaction to a foreign body in the submucosa, probably also linked to the surgery, were observed. Menchaca et al. analysed the histological changes in the serosa of the stomach of dogs subjected to gastric plication depending on the various types of anchorage used for the plication. Analysis of gastric mucosa revealed inflammatory changes and fibrosis predominantly in the suture and anchorage zones [7]. The above results, which agree with our findings in this study, suggest that in the long term gastric plication may lead to degeneration of the

stomach tissues or complications that are yet unknown because few studies of the technique have been carried out in this area.

Limitations: This is an experimental study. Unlike the findings we report here, some studies conducted with Zucker diabetic fatty rats have found that the two techniques are equally efficient [6]. The chosen animal model is therefore of great importance since a different model may produce a different response to surgery. To transfer this phenomenon to the clinical sphere, results must be analysed in accordance with the metabolic profile of each patient cohort. This is the only way reliable recommendations can be made. Results from experimental and clinical studies must also be analysed in the long term in order to determine the real effects of this type of surgery on weight and metabolism. To verify that the result of the SG is due to the decrease in intake, we would have to feed the sham group with the same amount of food that the SG group ingested. Therefore, experimental designs that are more adjusted to these results are necessary.

Conclusions: GP is not as effective as SG in terms of weight loss and metabolic control. The animals subjected to a GP continued to have a high appetite for the cafeteria diet unlike the animals submitted to an SG. Hormonal mechanisms possibly related to glucagon and ghrelin may be involved in this metabolic response.

COMPLIANCE WITH ETHICAL STANDARDS: All procedures performed are in accordance with the experimental ethical standards of the institutional research committee (Generalitat de Catalunya-registration number 3522).

CONFLICT OF INTEREST: The authors declare that they have no conflict of interest.

TABLES

Table 1

	Gastric Plication	Sleeve Gastrectomy	Sham
Weight increments weeks 13-16	113.58±23.9	82.33±21.6*	101.5±12.0
Intake week 12 (Kcal/day)	168.62 ± 19.5	170.88 ± 13.4	157.27 ± 14.1
Intake week 16 (Kcal/day)	146.03 ± 16.9*	134.43 ± 13.3*	161.88 ± 1.6

*p<0.05

Table 2

GLUC (mg/dl) χ±DS	**Week 8**	**Week 10**	**Week 12**	**Week 14**	**Week 16**	**p (weeks 12-16)**
Gastric Plication	99.75 ± 20.6	134.41 ± 15.8	180.91 ± 57.5	82.58 ± 20.9	162.91 ± 37.1	ns
Sleeve Gastrectomy	101.75 ± 7.7	147.33 ± 32.8	177.5 ± 66.8	95.08 ± 14.5	79.5 ± 14.3	p<0.05
Sham	101.37 ± 9.3	175.16 ± 37.5	184.87 ± 27.8	211.5 ± 52.5	165.37 ± 16.4	ns

Table 3

	Week 12			**Week 16**		
	Gastric Plication	**Sleeve Gastrectomy**	**Sham**	**Gastric Plication**	**Sleeve Gastrectomy**	**Sham**
Ghrelin ng/ml	1.14 ± 0.3	1.29 ±0.3	0.99 ±0.3	2.29 ±0.5*	1.070± 0.4**	1.344 ±0.1
Insulin pg/ml	5314.91 ±1877.1	5215.02 ±1613.9	4857.67 ±1137.0	3602.32 ±1216.4	2723.26 ± 973.2	3727.23 ± 2056.3
Glucagon pg/ml	34.24 ± 19.4	39.75 ± 10.5	46.56 ± 16.1	62.71 ± 36.2*	24.63 ± 9.3*/**	36.83 ± 12.3
GLP-1 pmol/L	36.17 ± 21.9	31.25 ± 11.1	26.81 ± 10.3	40.17 ± 24.5**	24.25 ± 10.3	20.81 ± 11.6
PYY pg/ml	91.68 ±51.1	84.73 ±58.1	52.06 ±22.5	97.52 ±83.8	132.24 ±98.7*	48.41 ±20.7

*p<0.05 between weeks

**p<0.05 between groups

FIGURE LEGENDS

Figure 1: Timeline of the experiment

Figure 2: A: Weight evolution in surgical groups, B: Weight evolution of each animal, C: Glycaemic evolution of each animal

Figure 3: Differences in intake (cafeteria diet and standard feed) according to groups and weeks

Figure 4: Differences in blood glucose according to groups and weeks

Figure 5: Sections of gastric biopsy after experiment. 5-A: GP group. 5-B: SG group. 5-C: Sham group.

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Figure 1: Timeline of the experiment

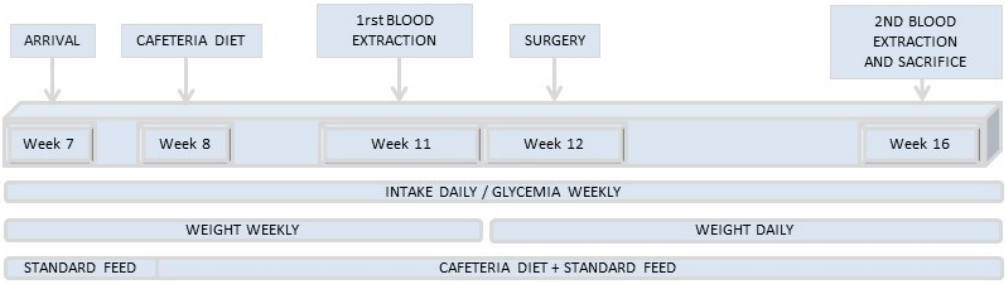

Figure 2: A: Weight evolution in surgical groups, B: Weight evolution of each animal, C: Glycaemic evolution of each animal

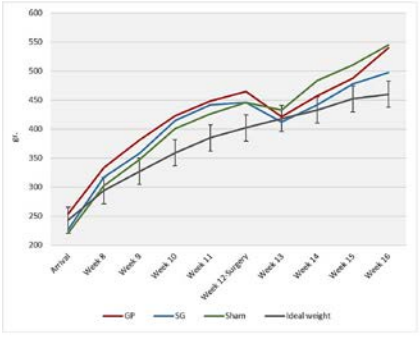

Figure 2A

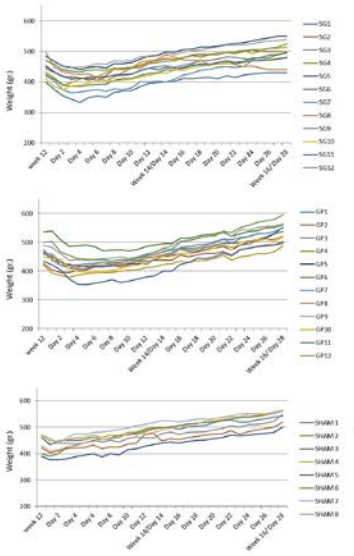

Figure 2B

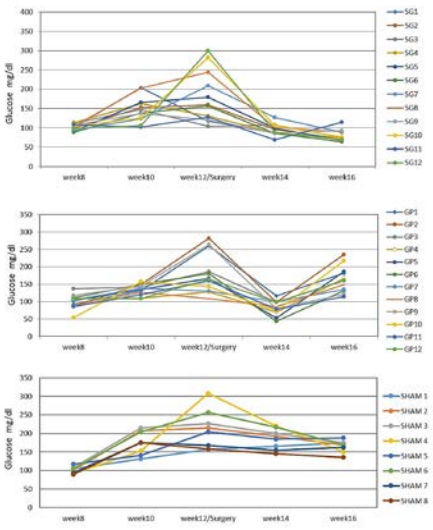

Figure 2C

Figure 3: Differences in intake (cafeteria diet and standard feed) according to groups and weeks

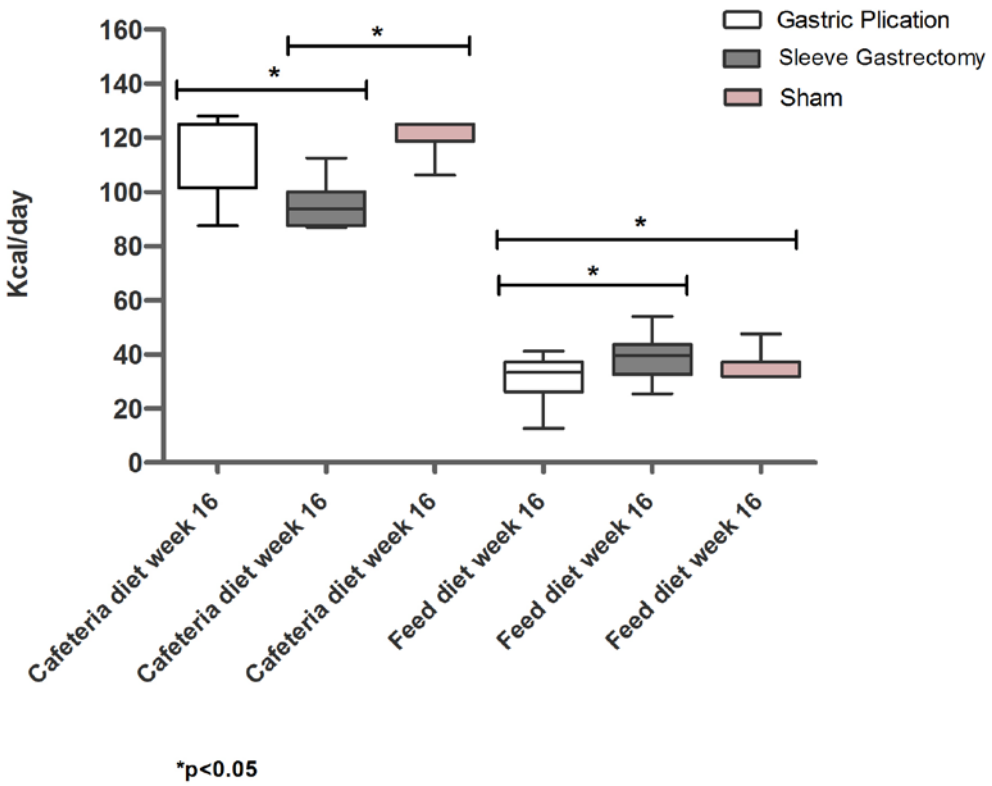

Figure 4: Differences in blood glucose according to groups and weeks

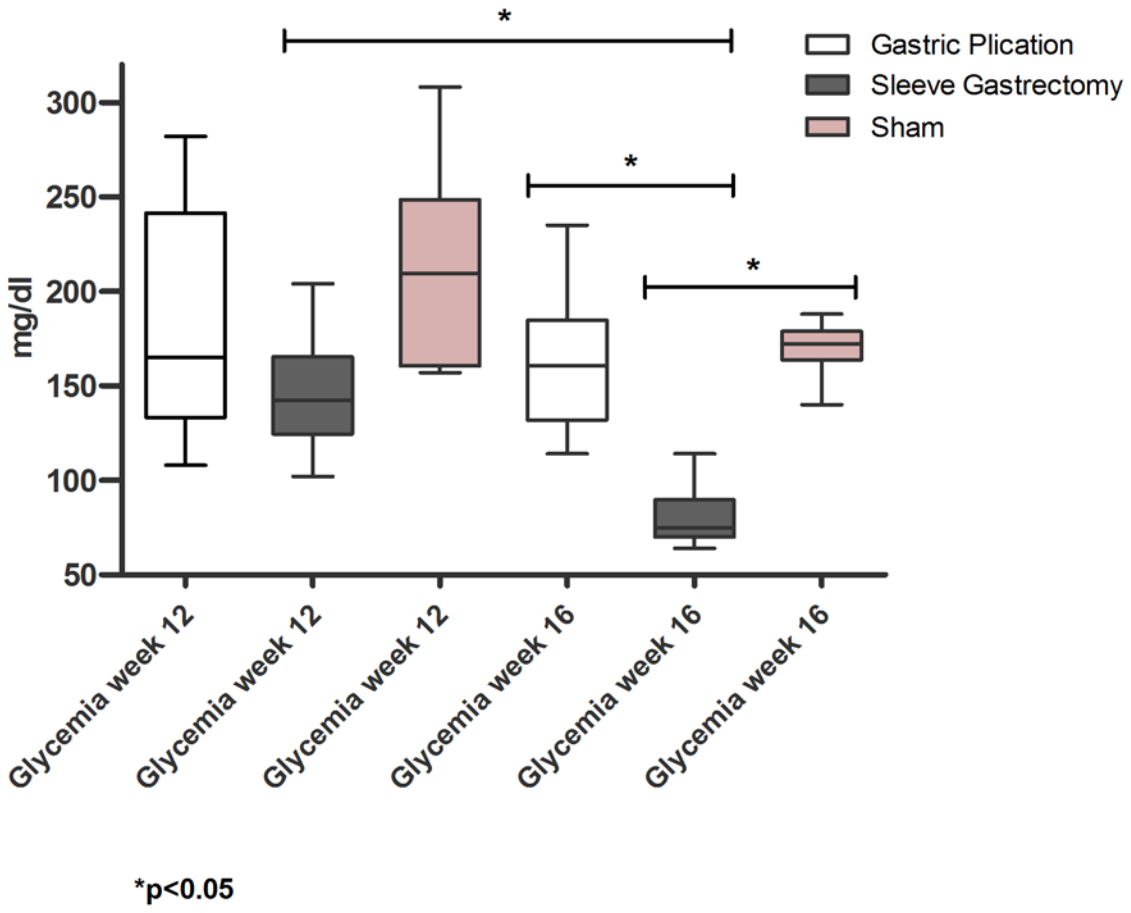

Figure 5: Sections of gastric biopsy after experiment. 5-A: GP group. 5-B: SG group. 5-C: Sham group.