ORIGINAL ARTICLE

CONTENTS

Adaptation of the small intestinal mucosa after single anastomosis gastric bypass

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ABSTRACT

Aim: The aim of our study was to perform morphological and morphometric analysis of biopsy specimens of the common and biliopancreatic loops after gastric bypass with a single anastomosis 3, 12, 24 months after surgery, which included measurement of villi length, ratio of villi length to the thickness of the lamina propria layer containing crypts, estimation of the number and distribution of goblet cells, quantification of number crypts and Paneth cells and comparing the changes in the biliopancreatic and common loops.

Materials and Methods: This study included 36 patients who underwent bariatric surgery due to morbid obesity. Patients underwent one of the following procedures: long-loop gastric bypass with one anastomosis, distal gastric bypass with one anastomosis, or mini-gastric bypass. Patients underwent EGDS with mucosal biopsy from the common and biliopancreatic loop at 3, 12, 24 months after gastric bypass with one anastomosis, followed by morphologic and morphometric study of biopsy specimens, which was part of our study.

Results: 2 years follow up show statistically significant differences in villus length were observed between the common and biliopancreatic limbs, with the length being greater in the common limb (0.390 ± 0.199 mm) than in the biliopancreatic limb (0.377 ± 0.184 mm) ($p < 0.05$). These changes may indicate hypertrophy of the villi in the efferent limb to increase the absorptive surface area. The thickness of the basal layer was greater in the biliopancreatic limb than in the common limb, measuring 0.196 ± 0.068 mm versus 0.167 ± 0.043 mm, respectively ($p< 0.05$). Regulatory functions of Paneth cells were preserved in both groups.

Conclusions: Adaptation of the small intestinal mucosa occurs after gastric bypass with one anastomosis, and these changes are more pronounced in the common loop of the small intestine. The regulatory functions of Paneth cells and their number involve both the common loop and the biliopancreatic region.

KEY WORDS: Obesity, one anastomosis gastric bypass, intestinal adaptation, small intestine villi, morphometry

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INTRODUCTION

Physiological adaptation in the small intestine is a continuous process of self-renewal through crypt cell proliferation, migration, and differentiation into specialized mucosal cells (such as enterocytes, enteroendocrine cells, goblet cells, or Paneth cells) [1]. Postresectional adaptation of the intestine is a natural compensatory process that leads to structural and functional changes in the intestine, improving the absorption of nutrients and fluids in the remaining functional section of the gut.

Structural adaptation of the intestine includes morphological and morphometric changes in the intestinal wall, such as mucosal hyperplasia, angiogenesis, elongation, and dilation of the remaining intestine [2]. Functional adaptation involves accelerated differentiation of crypt cells, delayed transit time, and increased expression of transporter proteins and exchangers involved in the absorption of nutrients, electrolytes, and water.

In adults, data on adaptive changes are rare, and the mechanisms underlying intestinal adaptation are not fully understood. Factors influencing the degree of intestinal adaptation after resection include the site and extent of resection, stimulation of the intestinal lumen by enteral nutrients, and intestinotrophic factors. Two intestinotrophic growth factors - teduglutide, a glucagon-like peptide II analog, and recombinant growth hormone (somatotropin) - have now been approved for clinical use in patients with short bowel syndrome. Both Of these medications enhance fluid absorption and reduce the need for parenteral nutrition and/or intravenous fluid administration. It is believed that intestinal adaptation in humans is limited to the first 1–2 years after resection.

Enterocyte apoptosis typically occurs in the remaining intestine due to increased crypt cell proliferation and is not considered to be inloved in adaptation mechanisms [3]. Therefore, most of the literature on adaptive and

Table 1. Characteristics of the study group

Variable	Results (n=36)
Sex	M: 15(41.7%); F: 21(58.3%)
Age (year)	47,9±10,6
Body mass index (kg/m²)	54.07 ± 8.8
%EWL (1, 2 years follow up)	1 year - 65± 2,6; 2 years- 67±1,1

Source: compiled by the authors of this study

Table 2. Comorbid diseases in general group (n=36)

Comorbidity	Patients with comorbidities n (%)
Type II diabetes mellitus	5(13.9)
Prediabetes	7(19.4)
Hyperlipidemia	25(69.4)
Hypertension	26(72.2)
Sleep apnea	12(33.3)
Osteoarthritis	16(44.4)

Source: compiled by the authors of this study

morphometric changes in the small intestinal mucosa has focused on treating short bowel syndrome.

Data on potential adaptive changes in the intestine after gastric bypass surgery primarily involve studies on glucose metabolism [4], experimental research on intestinal adaptation after gastric bypass in rats [5], and comparisons with massive resection influenced by glutamine [6]. We found studies examining lipogenesis in the jejunum to be more pronounced in patients with remission of diabetes after Roux-en-Y gastric bypass surgery. Another study that investigated the processes of intestinal adaptation was devoted to the study of intestinal permeability in which lactulose, mannitol ratio and lactulose excretion rate were evaluated. Increased mucosal permeability, according to the authors, was due to hyperplasia of the small intestinal mucosa in patients after bypass surgery [7,8].

However, no studies have been found specifically examining the morphological and morphometric changes in the small intestinal mucosa after single anastomosis gastric bypass in humans.

AIM

The aim of our study was to perform morphological and morphometric analysis of biopsy specimens of the common and biliopancreatic loops after gastric bypass with a single anastomosis 3, 12, 24 months after surgery, which included measurement of villi length, ratio of villi length to the thickness of the lamina propria layer containing crypts, estimation of the number and distribution of goblet cells, quantification of number crypts and Paneth cells and comparing the changes in the biliopancreatic and common loops.

MATERIALS AND METHODS

PATIENTS

The study included 36 patients who underwent bariatric surgery at the Thoraco-Abdominal Surgery Department of the State Institution "National Institute of Surgery and Transplantation named after O.O. Shalimov" of the National Academy of Medical Sciences of Ukraine due to morbid obesity. These patients received either long-loop gastric bypass with one anastomosis with a 200-cm biliopancreatic loop, distal gastric bypass with one anastomosis with a 250-cm common loop., or mini-gastric bypass with a 200-cm biliopancreatic loop between 2016 and 2022.

Inclusion criteria were patients who underwent long-loop gastric bypass with one anastomosis, distal gastric bypass with one anastomosis, mini-gastric bypass followed by endoscopic examination with biopsy at 3, 12, 24 months after surgery. Patients who had peptic ulcer disease were excluded from the study. Patients who had peptic ulcer disease were excluded from the study.

Characteristics of the study group presented in Table 1, Table 2.

Comorbid diseases are presented in Table 2.

A BRIEF DESCRIPTION OF THE SURGICAL TECHNIQUE OF GASTRIC BYPASS

The MGB technique was performed according to the standard method R. Rutledge with a 200-cm long biliopancreatic loop.

Long-loop gastric bypass with one anastomosis involves horizontal gastric transection at the border of the antrum and body using 2-3 non-articulating 60 mm blue loads (Covidien Endo GIA). After the gas-

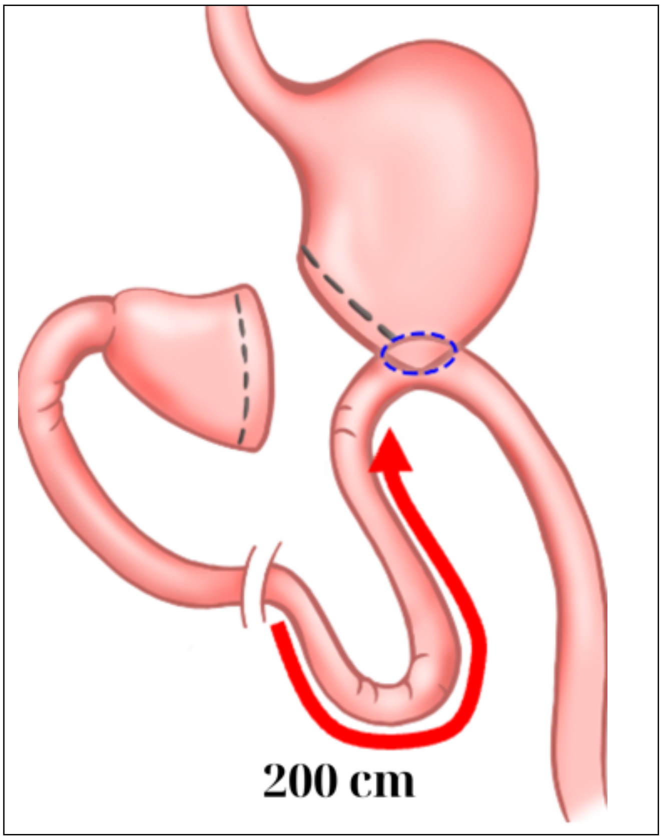

Fig. 1. Scheme of long - loop gastric bypass with one anastomosis
Source: compiled by the authors of this study

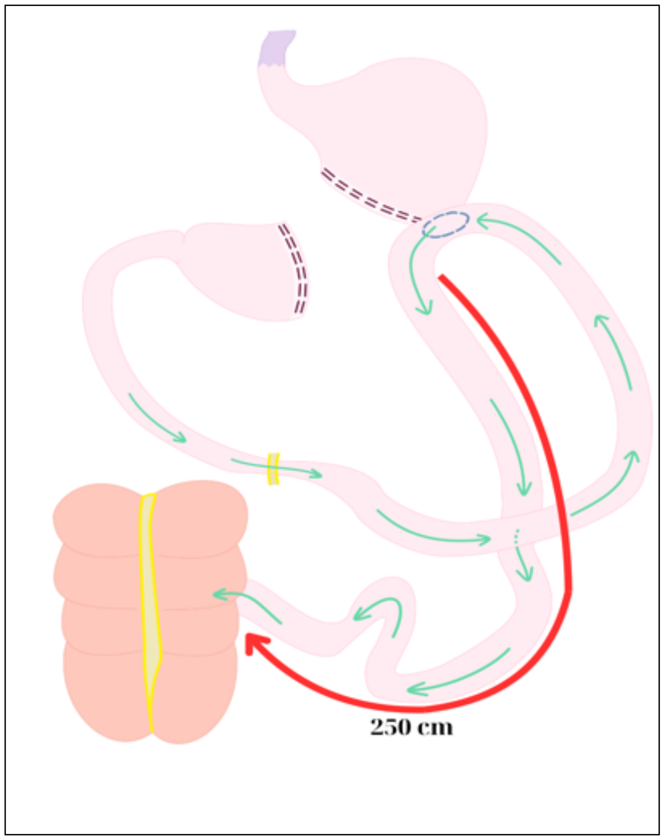

Fig. 2. Scheme of distal gastric bypass with one anastomosis
Source: compiled by the authors of this study

tric transection, 200 cm of the biliopancreatic loop is measured distal to the ligament of Treitz (Fig. 1). The gastrojejunostomy is formed using a single 45 mm non-articulating load through the angle of the staple suture along the posterior wall of the stomach at an angle of 90° to the line of horizontal gastric transection. Final closure of the gastrojejunostomy is hand sewn.

The distal gastric bypass with one anastomosis involves a similar gastric reservoir as the long-loop gastric bypass with one anastomosis. After transection of the stomach, 250 cm of the common loop is measured proximally from the ileocecal valve (Fig. 2). Gastrojejunostomy technique, closure of the gastrojejunostomy was done using a similar technique.

In all cases, the total measurement of the small intestine was not performed.

ENDOSCOPIC TECHNIQUE

Examinations were conducted using an Olympus GIF-EZ 1500 device. After endoscopic inspection of the esophagus and gastric stump, the mucosa of the biliopancreatic and common limbs was visually assessed 35–40 cm distal to the gastroenterostomy site, followed by forceps biopsy of the mucosa, with 1–2 samples taken from each section of the small intestine (Fig. 3, Fig. 4). The samples were placed in 10% formalin solution within 10 minutes and sent to the pathology department for further analysis. The endoscopic examination was performed by one person.

MORPHOLOGICAL STUDIES

Preparation for histological examination was carried out using standard methodology: after fixation of tissue fragments in neutral buffered 10% formalin, they were processed according to generally accepted histological techniques to obtain paraffin blocks. From the obtained paraffin blocks, 5 μm thick tissue sections were prepared and stained with hematoxylin-eosin according to standard procedures.

Two slides containing 2 to 6 sections were prepared for more detailed microscopic examination of the material. For description and morphometry, one section was selected that was free of technical defects and contained the largest area of the muscularis mucosa and the overlying propria and villi in maximum quantity. Histological examination was performed using an Olympus BX41 microscope, and morphometric analysis was conducted with an Olympus EP50 camera connected to the microscope and EPview software (Fig.5).

During the histological examination, a detailed description of the biopsy specimens of the mucous membrane of the biliopancreatic and common loop was performed.

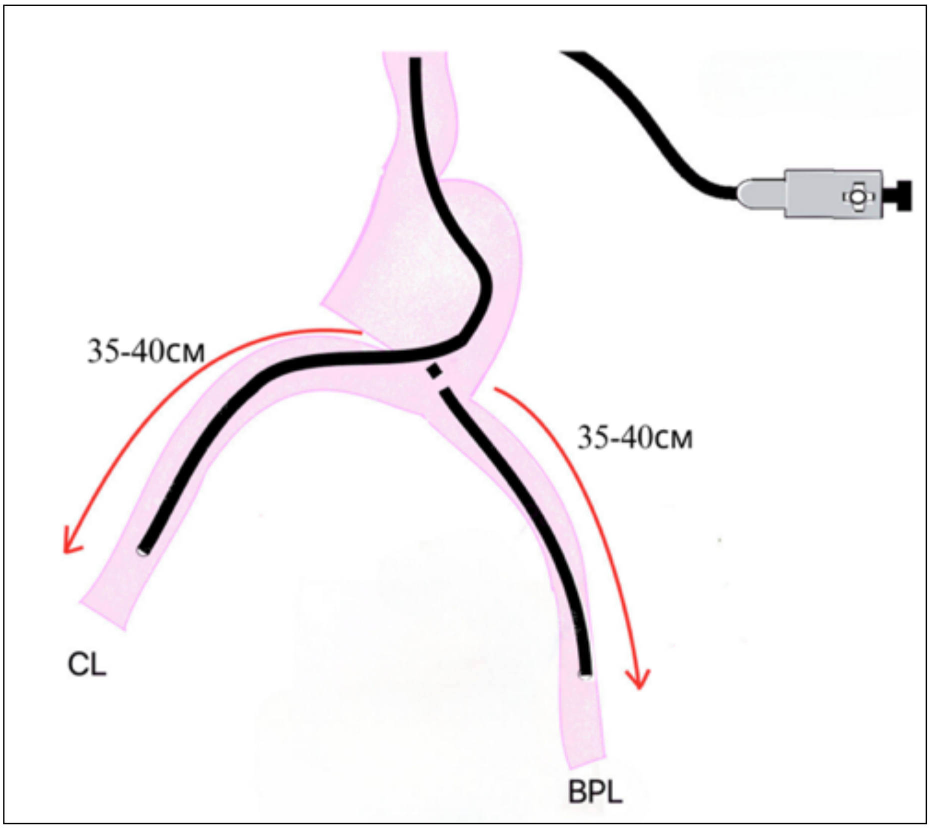

Fig. 3. Schematic representation of endoscopic forceps biopsy of the mucosa from the biliopancreatic limb (BPL) and common limb (CL) of the small intestine
Source: compiled by the authors of this study

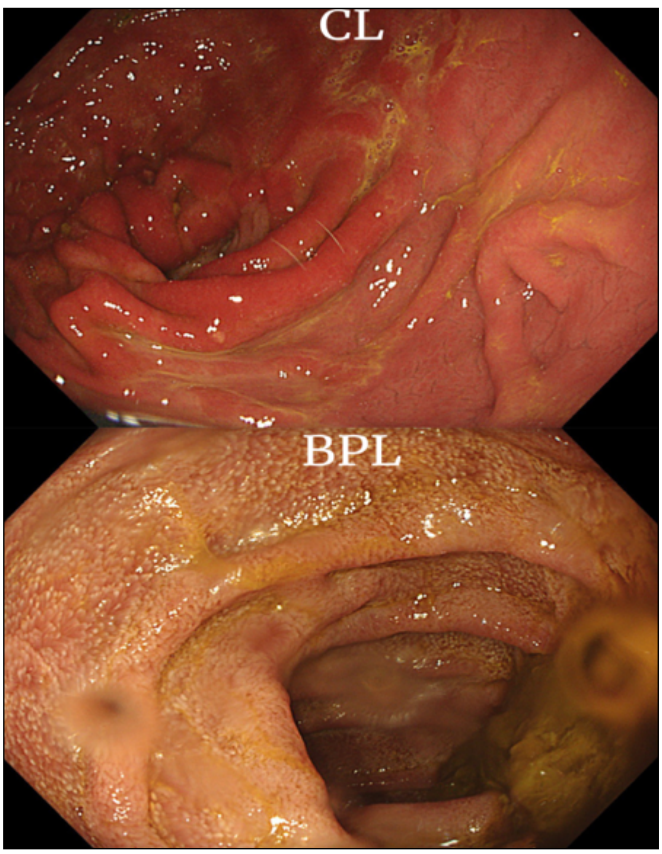

Fig. 4. Endoscopic view of the common limb (CL) and biliopancreatic limb (BPL) in a patient 3 years after single anastomosis gastric bypass surgery. Thickened folds of the mucosa are observed in the common limb
Picture taken by the authors

Morphometric analysis included measuring the length of the villi, the ratio of the length of the villi to the thickness of the layer of the lamina propria containing the crypts, assessing the number and distribution of goblet cells, and counting the number of crypts and Paneth cells.

ETHICS

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards (Ethics Committee of National Institute of Surgery and Transplantology named after A.A. Shalimov of the National Academy of Medical Sciences of Ukraine №16/01/2016).

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Informed consent was obtained from all individual participants included in the study.

RESULTS

LENGTH OF VILLI AND THICKNESS OF THE BASAL LAYER

The main function of intestinal villi in the small intestine is to directly and indirectly increase the absorption area,

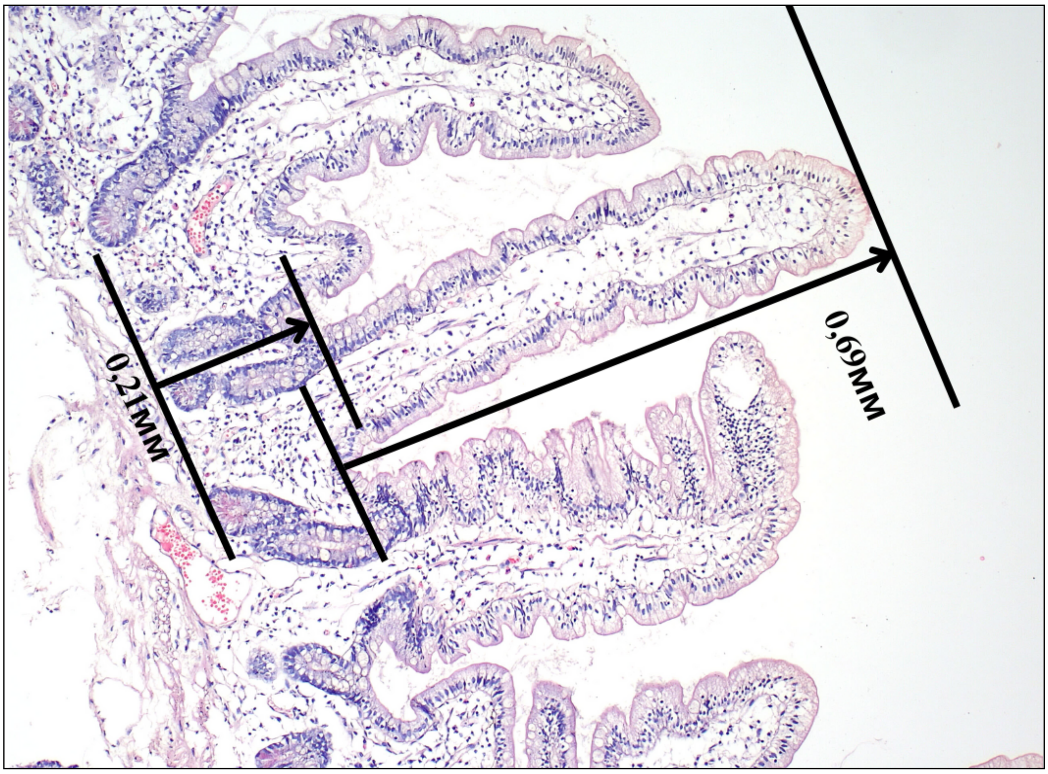

Fig. 5. Morphometry. Measurements of basal layer thickness (0.21 mm) and intestinal villus height (0.69 mm) were performed. Common loop with uneven villus expansion in the patient 14 months after mini-gastric bypass surgery. Magnification, 100X. Hematoxylin-eosin staging
Picture taken by the authors

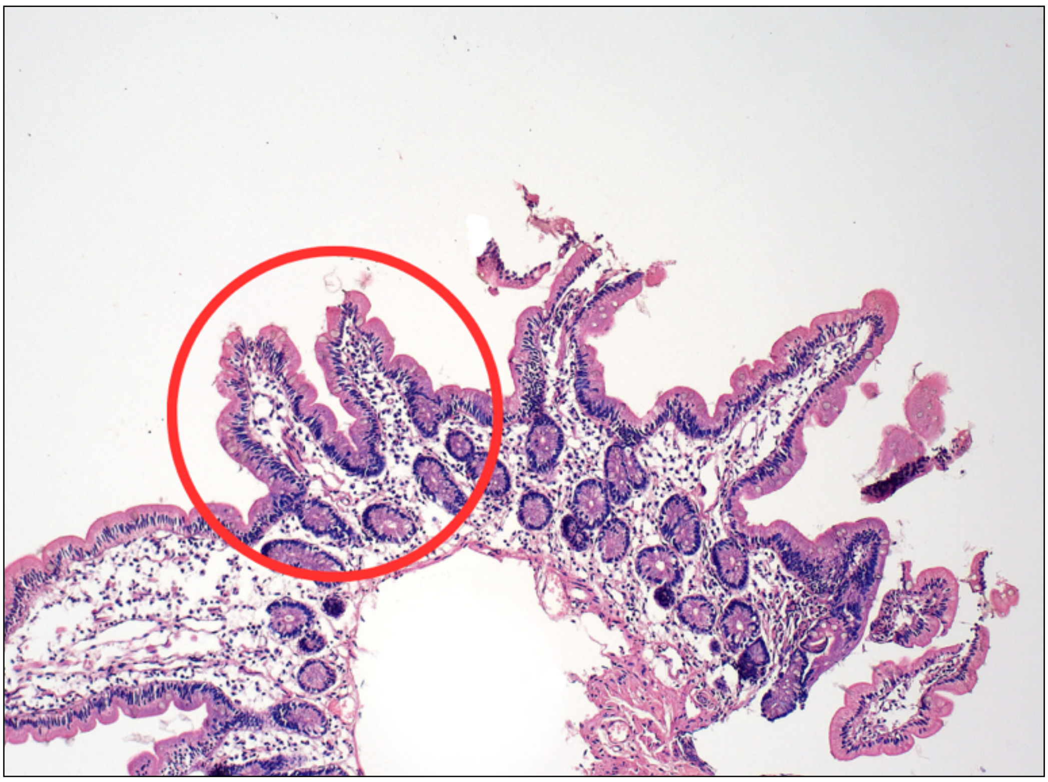

Fig. 6. A section of the mucous membrane of the biliopancreatic loop with flattening of the villi. Magnification, 100. Hematoxylin-eosin staining
Picture taken by the authors

thereby shortening the distance for nutrient molecules to reach the bloodstream and lymphatic vessels. **:** At 3 months follow up, no morphological or morphometric changes were observed in the biopsies from the biliopancreatic or common limbs. The first morphological and morphometric changes in the small intestinal mucosa were noted at 12 months after surgery. The results show the following changes in the mucosal biopsy after 2 years follow up. A total of 504 villi were studied in 36 patients. For all 36 patients, the average length of the villi in the biliopancreatic and common loops was calculated (see table). Subsequently, these indicators were compared, and statistically significant differences in the length of villi and the thickness of the basal layer

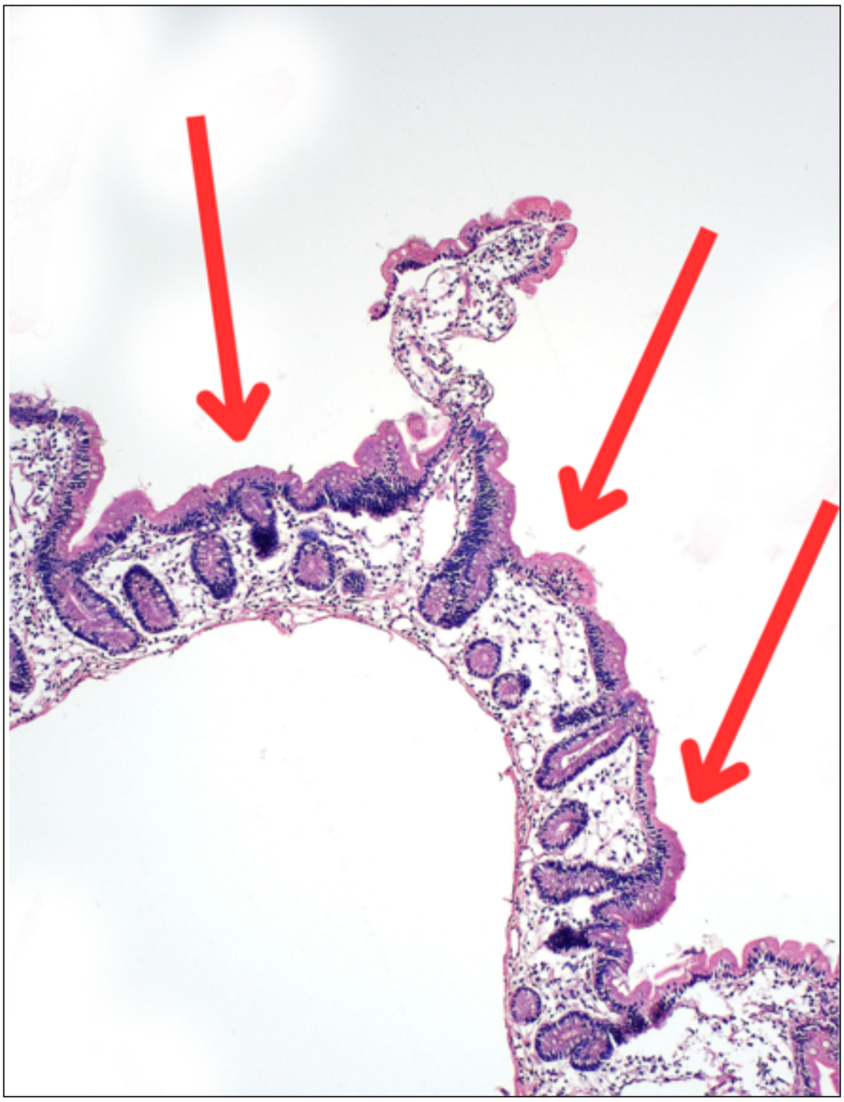

Fig. 7. Mucosal area with shortened villi. Magnification, 100X. Hematoxylin-eosin staining
Picture taken by the authors

between the common and biliopancreatic loops were analyzed using the non-parametric Mann-Whitney U-test. (Table 3).

A statistically significant ($p < 0.05$) difference in villi length was noted between the common and biliopancreatic loops of the small intestine, with the villi being longer in the common loop (0.390 ± 0.199 mm) compared to the biliopancreatic loop (0.377 ± 0.184 mm). These changes suggest villi hypertrophy in the efferent loop of the intestine to enhance the absorption area. This is also indicated by the prevalence of tortuous, unevenly expanded, and branched villi over shortened, deformed, and fused ones, which may correspond to structural intestinal adaptation (Fig. 6, Fig. 7).

The underlying cause of these changes is the reduction in the functional area of the small intestine, where absorption occurs—the area of the common loop—during surgery, as well as the body's need to compensate for postoperative malabsorption, which is the goal of the surgical intervention. In contrast, in the biliopancreatic loop, there is no need for absorption processes due to the absence of chyme, leading to villi atrophy. These results fully align with these hypotheses.

Statistically significant ($p < 0.05$) differences were also found in the average thickness of the basal layer between the common and biliopancreatic loops, with greater thickness observed in the biliopancreatic loop (0.196 ± 0.068 mm) compared to the common loop (0.167 ± 0.043 mm).

QUANTIFICATION OF CRYPTS AND PANETH CELLS

Paneth cells and crypts perform two primary functions: producing α-defensins and lysozyme to protect the body from microorganisms in the intestinal lumen, and regulating apoptosis and necroptosis in the intestinal mucosa. Crypts were identified as any closed tubular structures within the thickness of the basal layer of the mucosa, except for those where tangential sections only revealed cell nuclei.

The following results were obtained from observing the number of crypts and Paneth cells (Table 4).

Table 3. Villus length, basal layer thickness of the biliopancreatic (BPL) and common loop (CL) layers and the mean ratio were determined

Average pile length, mm			Average thickness of the basal layer, mm			Correlation		
CL	BPL	Mann-Whitney U test.	CL	BPL	Mann-Whitney U test.	CL	BPL	Mann-Whitney U test.
0.390± 0.199	0.377± 0.184	p<0.05*	0.167± 0.043	0.196± 0.068	p<0.05*	1.927± 1.30	2.342± 1.07	p<0.05*

* - Nonparametric Mann-Whitney test
Source: compiled by the authors of this study

Table 4. Average values of the total number of crypts and crypts containing Paneth cells

Biliopancreatic Loop		Common Loop		p
Crypts total, pcs.	Crypts containing Paneth cells, pcs.	Crypts total, pcs.	Crypts containing Paneth cells, pcs.	
54.2±14.8	19.7±7.2	52.8±13.8	18.2±7.1	>0.05 *

* - Nonparametric Mann-Whitney U test
Source: compiled by the authors of this study

Table 5. Results for goblet cell count and their distribution in the epithelial lining

Loop Type	Patients with Discrete Goblet Cell Distribution	Goblet Cell Percentage in Epithelium	Patients with Physiological Goblet Cell Distribution	Goblet Cell Percentage in Epithelium
Biliopancreatic	18 (50%)	10%	18 (50%)	25.9%
Common	0 (0%)	–	36 (100%)	18.2%

Source: compiled by the authors of this study

The average total number of crypts in the biliopancreatic loop was slightly higher than in the common loop (54.2 ± 14.8 vs. 52.8 ± 13.8, respectively), but the Mann-Whitney U-test depicted no statistically significant differences ($p > 0.05$). Therefore, the number of crypts in the biliopancreatic and common loops can be considered comparable.

Similarly, the number of crypts containing Paneth cells was slightly higher in the biliopancreatic loop compared to the common loop (19.7 ± 7.2 vs. 18.2 ± 7.1, respectively). However, no statistically significant differences were found using the Mann-Whitney U-test ($p > 0.05$). Thus, the number of crypts containing Paneth cells can also be considered comparable. These findings suggest that the regulatory functions of Paneth cells are preserved regardless of the type of single-anastomosis gastric bypass performed.

ASSESSMENT OF GOBLET CELL COUNT AND DISTRIBUTION

Goblet cells exhibit physiological characteristics in their distribution within the small intestine: their count decreases from the base to the tip of the villus and increases from the duodenum to the ileum.

In the small intestine, goblet cells perform mucin secretion through two mechanisms: regulated vesicular secretion and compound exocytosis.

1. Regulated secretion involves individual secretory vesicles merging with the plasma membrane to release their contents.
2. Compound exocytosis entails intracellular vesicle fusion followed by the "bursting" of the entire cell and the release of its contents into the intestinal lumen.

Given the migration of cells from crypts to the villus tip, fewer mature goblet cells (those that have not yet "burst") are found at the tip, while younger cells are concentrated at the base.

The observations yielded the following results for goblet cell count and their distribution in the epithelial lining (Table 5).

Interestingly, the distribution of goblet cells in the mucosa of the biliopancreatic and common loops differed significantly. In the biliopancreatic loop mucosa, half of the patients exhibited discrete goblet cell distribution, while the other half showed physiological distribution. For discrete distribution, goblet cells accounted for 10% of all epithelial cells on average, while physiological distribution yielded an average of 25.9%.

In the common loop mucosa, all 36 patients demonstrated preserved physiological goblet cell distribution, with goblet cells accounting for an average of 18.2% of all epithelial cells. These findings may reflect adaptation to environmental factors, such as altered microbiota or local influences affecting mucin secretion. A higher concentration of bile and pancreatic juices, undiluted by chyme, may negatively impact the epithelial lining, necessitating compensation through increased mucin production for protective functions.

The differing goblet cell distribution patterns and counts may be associated with the predominance of different mucin secretion mechanisms. However, these findings require further investigation to understand the regulatory mechanisms of goblet cell function fully.

DISCUSSION

The mucosa of the biliopancreatic limb, excluded from the digestive process, is constantly exposed to digestive juices, including secretions from the bypassed gastric reservoir, the pancreas, and bile. Unlike those in the common limb, luminal and membrane digestion and absorption of micronutrients and nutrients do not occur in the biliopancreatic limb, leading to impaired nutrition of the enterocytes themselves.

Considering these processes, the following adaptive changes in the mucosa of the biliopancreatic limb of the small intestine can be predicted [10]:

1) The villi were flattened, and the mucosal surface was smoothed to reduce the contact area with irritants;

2) An increase in the number of goblet cells producing mucins to enhance the protective function against digestive juices;

3) The number of Paneth cells in the crypts decreases as the need for α-defensins and lysozyme production decrease. However, since these cells still play a role in modulating apoptosis and necroptosis, this change is less likely.

4) Increased chronic inflammatory infiltration, mucosal erosion, and focal fibrosis in areas of continuous damage to the intestinal wall.

The common limb of the small intestine retains all of its functions -digestion, absorption, motility, and evacuation - and is in contact with digestive juices mixed with chyme. However, due to the reduced surface area of the small intestine following gastric bypass surgery, the following changes can be predicted:

1) The enlargement of villi, branching, and deformation occur, with uneven widening to increase the surface area available or contact with chyme;

2) Hypertrophy of the epithelium, especially enterocytes, to enhance digestive activity and absorption;

3) The enlargement of lymphatic capillaries in the lamina propria occurs as a result of increased absorption.

For a more objective interpretation of the morphological changes in the small intestine, the following microscopic and morphometric criteria were considered [11]:

1) Villi are elongated and finger-shaped;

2) The normal villus structure included at least four elongated, finger-shaped villi in succession;

3) The ratio of villus length to the thickness of the lamina propria containing crypts should be 3-5:1;

4) The surface is covered with microvilli that visually form an eosinophilic brush border, allowing for the identification of enterocyte damage during erosion;

5) The number of intraepithelial lymphocytes (IELs) is 1 for every 5 enterocytes;

6) The number of goblet cells decreases from the base to the tip of the villus, with a general increase from the duodenum to the ileum;

7) Paneth cells are located at the basal region of the crypts (lower 25%), and their number increases from the duodenum to the ileum;

8) The lamina propria contains loose connective tissue with minimal lymphoplasmacytic infiltration and occasional eosinophils.

Compared to the large number of animal studies investigating intestinal adaptation after gastric resection or gastric bypass surgery, the number of human studies is relatively small. The reasons for this discrepancy are the invasiveness of many procedures and the need to assess structural and functional adaptation [9]. For example, histologic analysis of the small intestine requires a biopsy, which can be particularly difficult for patients who have undergone multiple abdominal surgeries.

In our study, we compared the morphological changes in two types of intestinal loops-common and biliopancreatic - after single-anastomosis gastric bypass surgery, with a special focus on changes in villus length, basal layer thickness, and the number of crypts and Paneth cells. Our findings reveal that certain differences between these two types of loops may indicate adaptive changes in the intestine in response to gastric bypass surgery with a single anastomosis, which in turn may explain the stabilization of excess weight loss after 6-12 months of the postoperative period, "unsatisfactory" loss of excess weight, weight gain 2-3 years after surgery, or even regression of obesity and metabolic disorders in the longer-term postoperative period.

CONCLUSIONS

After 2 years follow-up the average length of the villi in the common loop was longer than that in the biliopancreatic loop which may indicate structural adaptation of the intestine, which increases the absorption area in the common loop. The thickness of the basal layer also tend increase in the biliopancreatic loop. Preservation of the number of Paneth cells in both loops indicates the constancy of their functions, regardless of morphological changes and changes in the anatomy of the gastrointestinal tract.

To further deepen our understanding of these processes, additional studies with a larger number of samples and the use of modern methods of morphological analysis are needed.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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