Effects of food and gastrointestinal secretions on intestinal integrity in an experimental rat model

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Abstract. The present study aimed to investigate the individual and combined effects of food and biliopancreatic secretions on intestinal morphology, tight junction (TJ) protein expression and microbial composition in a rat model. A total of 30 male Sprague-Dawley rats were randomly allocated into three groups: Control, biliopancreatic diversion and jejunal bypass. Jejunal segments were surgically manipulated to selectively exclude biliopancreatic secretions, food or both. Corresponding control segments were also collected. Intestinal samples were analysed for villus height/crypt depth ratios and intraepithelial lymphocyte (IEL) counts using H&E staining, as well as TJ protein expression (occludin, claudin-1, zonula occludens-3) measured using immunohistochemistry. In addition, portal venous blood samples were obtained to assess serum lipopolysaccharide and citrulline levels using ELISA. Furthermore, quantitative bacterial cultures were performed on jejunal tissues. The villus height/crypt depth ratio was significantly reduced in segments deprived of both bile and food (P=0.007), and IEL counts were significantly elevated in food- and bile-deficient segments (P<0.001). Moreover, claudin-1 expression was significantly lower in food-deficient segments (surgically created intestinal segment that is bypassed from luminal nutrient flow)(BPS1 and JPS2) (P=0.035), whereas occludin expression was increased in the presence of food (P=0.016). In conclusion both food and biliopancreatic secretions are essential in maintaining intestinal epithelial morphology and TJ integrity. Food deprivation induced more pronounced changes in intestinal integrity than the absence of biliopancreatic secretions. The present findings highlight the synergistic role of luminal nutrition and digestive secretions in preserving gut barrier function.

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Introduction

The gastrointestinal epithelium serves as a barrier between the external environment and the internal milieu of the body. This barrier is crucial for preventing the translocation of bacteria and toxins into systemic circulation (1). Intestinal permeability occurs via two main pathways: Paracellular and transcellular. The transcellular pathway involves the passage of molecules through the epithelial cells, via processes such as endocytosis, vesicular transport, and exocytosis, and is regulated by membrane transporters and channels. Paracellular permeability is governed by tight junction (TJ) proteins; TJs consist of several complex membrane proteins, including claudins, occludin and zonula occludens (ZO) (Fig. 1). Epithelial TJs can be dynamically modulated by various signals, including humoral and neural factors that engage multiple cellular pathways, and alterations in the expression of these proteins are implicated in various diseases such as inflammatory bowel and celiac disease, and irritable bowel syndrome (2,3).

Intestinal barrier function is influenced by numerous factors such as diet, stress, microbiota and drugs. Increased intestinal permeability has been associated with gastrointestinal disorders, including celiac disease, inflammatory bowel disease, irritable bowel syndrome and food allergies, as well as systemic conditions such as schizophrenia, multiple sclerosis, diabetes mellitus and sepsis (4). The mucosal barrier is essential for human health, and several strategies have been developed to strengthen this barrier. One such strategy is to maintain intestinal integrity through enteral nutrition, which refers to the delivery of nutrients directly into the gastrointestinal tract via oral intake or feeding tubes (nasogastric or gastrostomy tubes). Additional approaches include the use of probiotics and prebiotics to enhance TJ stability and support beneficial microbiota composition, as well as dietary fibers and short-chain fatty acids (5). Enteral nutrition preserves mucosal structure by providing luminal nutrients that stimulate epithelial cell turnover, enhance TJ protein expression, and support local

immune function. This strategy is commonly implemented in clinical and perioperative settings to prevent intestinal atrophy and maintain barrier integrity. Developing strategies such as this is important as compromised intestinal integrity can lead to endotoxemia and a proinflammatory state (6).

Bile and pancreatic secretions are important for digestion, particularly for fatty acid absorption, and contribute to cholesterol homeostasis. Furthermore, bile contributes to intestinal barrier function by modulating glucose and lipid metabolism, and may influence enterocyte proliferation and apoptosis (7). The present study aimed to evaluate the impact of the presence of luminal nutrients (food) and biliopancreatic secretions on intestinal integrity to guide clinical strategies in settings where maintaining intestinal barrier function is critical.

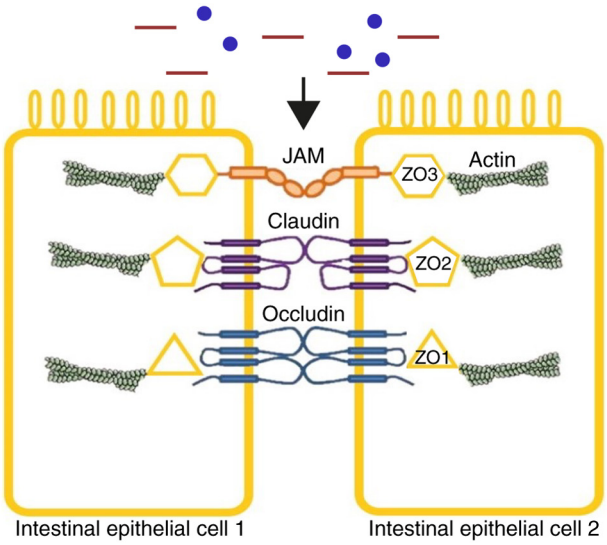

Figure 1. Schematic illustration of TJs in intestinal epithelial cells. TJs are depicted at the apical region between adjacent epithelial cells, forming a seal that regulates paracellular permeability. TJ, tight junctions; ZO, zonula occludens; JAM, junctional adhesion molecule

Materials and methods

Animals. A total of 30 adult male Sprague-Dawley rats (8-10 weeks; weight, 240-380 g) were housed under controlled conditions (22˚C; 50-60% humidity; 12-h light/dark cycle) and fed a standard diet (DSA Agrifood Products Inc.) with free access to both food and water for 10 days prior to surgery.

Animal groups and surgical design. Rats were randomly divided into three groups (n=10 each): i) Group 1 (control), laparotomy + two jejunal enterotomies and re-anastomoses; ii) group 2, biliopancreatic diversion (BP) with separated food and biliopancreatic secretion segments; and iii) group 3, jejunal bypass (JP) with food and biliary-deficient isolated jejunal segments.

Anaesthesia was induced with ketamine (40 mg/kg) and xylazine (10 mg/kg), and the skin was prepped with 10% povidone-iodine. Sterile conditions were maintained throughout. Prophylactic cefazolin (60 mg/kg) and subcutaneous morphine (1 mg/kg) were administered. All laparotomies were midline.

In group 1 (control), the jejunum was transected 30 and 90 cm distal to the ligament of Treitz, then re-anastomosed using 6-0 polydioxanone sutures (Fig. 2A). The abdomen was irrigated with saline and closed using standard techniques. In group 2 (BP), the duodenum was transected and closed; the jejunum was divided 20 cm from Treitz. The distal jejunum was anastomosed to the duodenum and the proximal segment was reconnected 40 cm downstream (Figs. 2B-3A). Anastomotic integrity was tested before closure (Video S1). In group 3 (JP), the jejunum was transected 30 cm from Treitz, and the proximal and distal ends were reconnected 40 cm apart (Fig. 2C). The bypassed segment was closed and fixed to the abdominal wall.

Once surgery was complete, the surgical sites were disinfected with chloramphenicol and iodine. Postoperatively, rats recovered for 30 min before being returned to individual cages. On day 1, a standard diet and water (with 100 mg/kg acetaminophen) was resumed. Body weights were recorded every 3 days. On day 24, animals were re-anesthetized using ketamine (40 mg/kg) and xylazine (10 mg/kg). Euthanasia was performed via exsanguination through portal vein puncture under deep anaesthesia. Cessation of heartbeat and respiration were used to confirm the death of all animals.

Sample collection. Blood samples were collected from the portal vein at the time of sacrifice. Intestinal tissue samples were then harvested immediately. Samples intended for microbiological analysis were processed fresh, while tissues for immunohistochemistry were fixed in formalin and embedded in paraffin blocks prior to staining. Samples were collected for histopathological and microbiological examination as follows: Control group (n=10): $Control_{S1}$, 10 cm from the ligament of Treitz; $control_{S2}$, 50 cm from the ligament of Treitz; $control_{S3}$, 80 cm from the ligament of Treitz (Fig. 2A). Group 2, BP (n=9): BP_{S1}, 10 cm from the ligament of Treitz, biliopancreatic secretion (+); BP_{S2}, 30 cm from the gastrojejunostomy, food (+); BP_{S3}, 20 cm from the jejunojejunostomy, representing the common limb through which both food and biliopancreatic secretions pass (Fig. 2B). Group 3, JP (n=8): JP_{S1}, 10 cm from the ligament of Treitz; JP_{S2}, 20 cm from the stump of the blind loop, biliopancreatic secretion (-) and food (-); JP_{S3}, 10 cm from the jejuno-jejunostomy, common intestinal limb (i.e., the segment where luminal contents and biliopancreatic secretions mix) (Fig. 2C). Analyses of histopathological and immunohistochemical findings were performed, comparing the intestinal segment samples to other segments within the same group and also to those of the control group (Fig. S1A).

Histopathology and immunohistochemistry. For histopathological examination, tissue samples were fixed in 10% neutral buffered formalin at room temperature for 24 h, embedded in paraffin blocks, and sectioned at 3 μm thickness. Sections were stained with hematoxylin (4 min) and eosin (30 sec) at room temperature. The stained slides were examined under a light microscope (Olympus SL-50). Villus height/crypt depth ratios were evaluated (2:1=atrophic, 5:1=normal) based on literature standards (8). Intraepithelial lymphocytes (IELs) counting was performed on H&E-stained sections. For each sample, four randomly selected high-power fields (x400 magnification) were evaluated. The number of intraepithelial lymphocytes was manually counted and expressed as the number of IELs/100 epithelial cells (≤20=normal, >20=high).

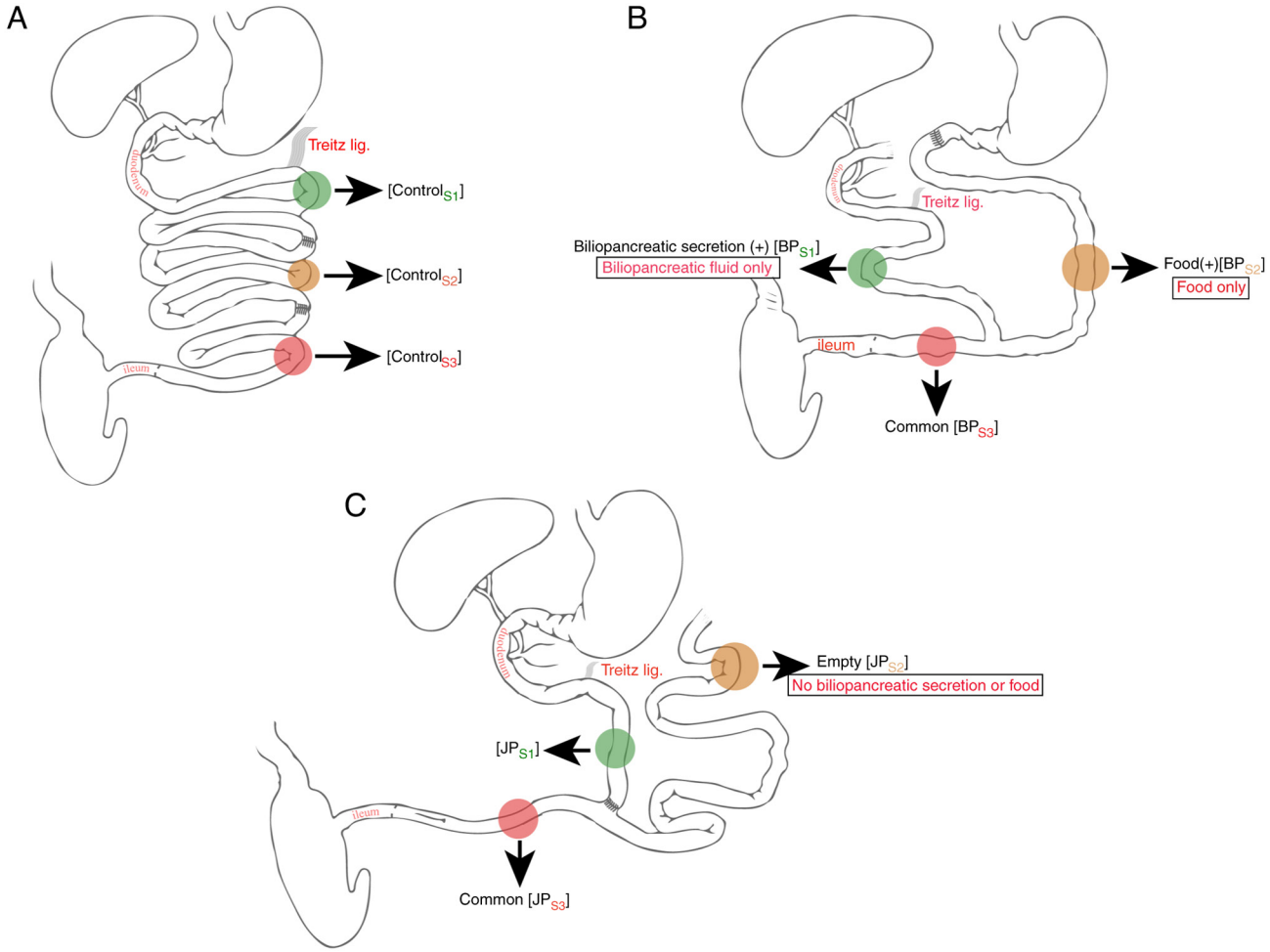

Figure 2. Overview of the experimental surgical design. Schematic illustration of surgical procedure and sampling for (A) control, (B) BP and (C) JP groups. BP, biliopancreatic diversion; JP, jejunal bypass; lig, ligation; S, segment.

IELs were identified as small, round intraepithelial cells with darkly stained, round nuclei and minimal cytoplasm. Neutrophils were excluded from IEL counts based on their characteristic multilobed nuclei and lighter cytoplasmic staining. Tissue samples were also stained for the TJ proteins occludin, claudin-1 and ZO-3. All immunohistochemistry examinations were performed on paraffin-embedded sections. Following deparaffinization, endogenous peroxidase activity was blocked with 3% hydrogen peroxide for 5 min for 5 min at room temperature. Non-specific binding was blocked using 5% normal goat serum (Sigma-Aldrich; Merck KGaA; cat. no. G9023) for 30 min at room temperature. Antigen retrieval was performed by boiling the sections in citrate buffer for claudin-1 or EDTA buffer for occludin and ZO-3 at 95˚C for 20 min. Sections were incubated with primary rabbit monoclonal antibodies at ~95˚C against claudin-1 (Abcam, cat. no. ab140349, 1:200), occludin (Abcam, ab168986, 1:150), and ZO-3 (Abcam, ab191143, 1:200) for 20 min. Detection was performed using the Leica HRP-conjugated detection kit (DS9800, New Castle, UK), followed by sequential incubation with 3% hydrogen peroxide (10 min at room temperature) and DAB (6 min at room temperature). Slides were counterstained with hematoxylin for 1-2 min at room temperature. Skin, kidney, and intestinal tissue were used as positive controls for claudin-1, occludin, and ZO-3, respectively.

Results were scored for staining intensity of occludin as weak (400x), moderate (100x), or strong (40x). Results were scored for staining intensity of ZO-3 as weak (400x), moderate (200x), or strong (40x). Results were scored for staining pattern of claudin-1 as weak (≤1/3 of villi) or strong (>1/3 of villi) (9) Staining intensity was evaluated semi-quantitatively based on the brown DAB chromogenic signal and the proportion of positively stained epithelial cells. All histopathological and immunohistochemical samples were initially evaluated by a pathologist. Subsequently, the samples were blindly reviewed by another pathologist.

Microbiological and biochemical analyses. After laparotomy and blood sampling, the intestinal segments were excised and immediately placed into pre-weighed sterile containers containing 0.9% normal saline. The containers were weighed again to determine the tissue mass by difference. Each tissue sample was then homogenized using a sterile tissue homogenizer in saline at a 1:10 (w/v) ratio. Ten-fold serial dilutions (10^{-1}-10^{-5}) were prepared, and 100 μl from each dilution was plated on blood agar and Endo agar. The plates were incubated aerobically at 37˚C for 24 h, after which colony numbers were counted. Bacterial load was expressed as colony-forming units per gram of tissue (CFU/g). Portal blood was sampled from the portal vein, as demonstrated in Fig. 3B, for the measurement of

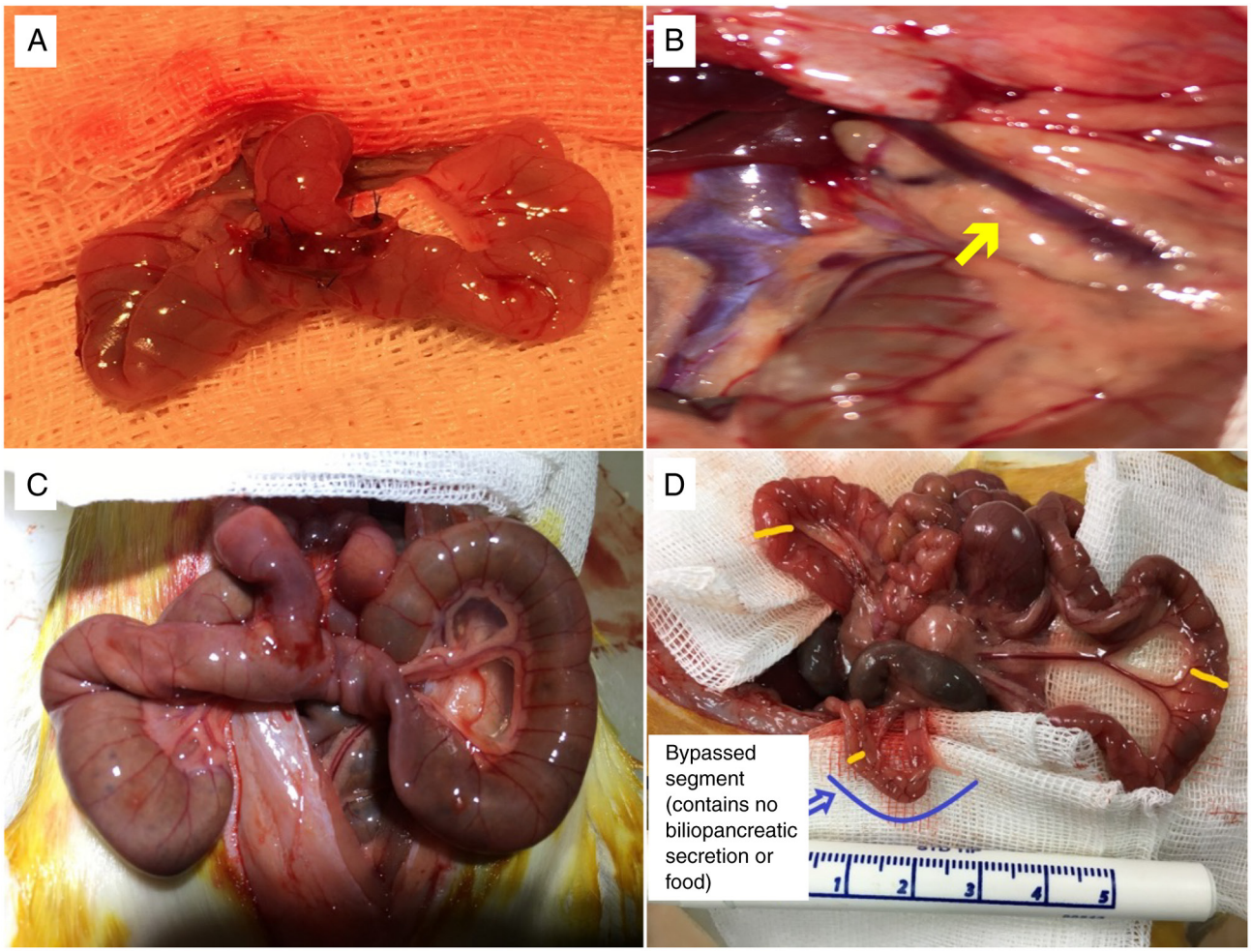

Figure 3. Macroscopic and procedural images of the experimental model. (A) completed jejunojejunostomy anastomosis, (B) blood sampling from the portal vein (arrow), (C) healed anastomosis before tissue collection, and (D) markedly atrophied bypassed segment.

plasma lipopolysaccharide (LPS) and citrulline levels. Plasma was isolated from heparinized portal blood samples and stored at -80˚C until analysis. Plasma concentrations of lipopolysaccharides were measured using an ELISA kit (Elabscience, cat. no: E-EL-0025), and plasma citrulline levels were measured using an ELISA kit (MyBioSource; cat. no. MBS2600386), according to the manufacturers' instructions.

Statistical analysis. Statistical analyses were performed using SPSS Statistics version 23 (IBM Corp.). Proportions of histopathological and immunohistochemical data (ordinal variables) were presented using cross-tabulations. χ^2 or Fisher's exact test, as appropriate, was used to compare the proportions in the different groups. The results of descriptive analyses are presented as the mean and standard deviation for weight and biochemical variables. The Kruskal-Wallis test was used to compare those parameters. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Macroscopic findings. Three animals (two from the JP group and one from the BP group) died spontaneously prior to day 24. Necropsy revealed major intra-abdominal complications consistent with leakage and infection, as the cause of death. These deaths were excluded from the final histopathological and biochemical analyses. Overall, no other major intra-abdominal complications (such as abscess or fluid accumulation) were observed in the animals used for analysis. Some animals had mild adhesions between bowel loops, but no obstruction or stenosis was detected. Gross intestinal atrophy was most evident in segments deprived of both food and biliopancreatic secretions (Fig. 3D).

Weight changes. All animals gained weight postoperatively, with no significant differences between the groups (Fig. S1B). The mean weights prior to euthanasia were 404±54 in the Control group, 361±65 g in the BP group, and 397±50 g in the JP group.

Villus/crypt ratio. Villus/crypt ratios were assessed to evaluate mucosal integrity across intestinal segments, and representative histological images are presented in Fig. 4A-C, E-F. When villus height/crypt depth ratios of the first segments (S1) in all groups were compared, a significant decrease in villus height/crypt depth ratio was observed in the BP_{S1} segment, where only biliopancreatic secretions were present. When the villus height/crypt depth ratio of the second segments (S2) of

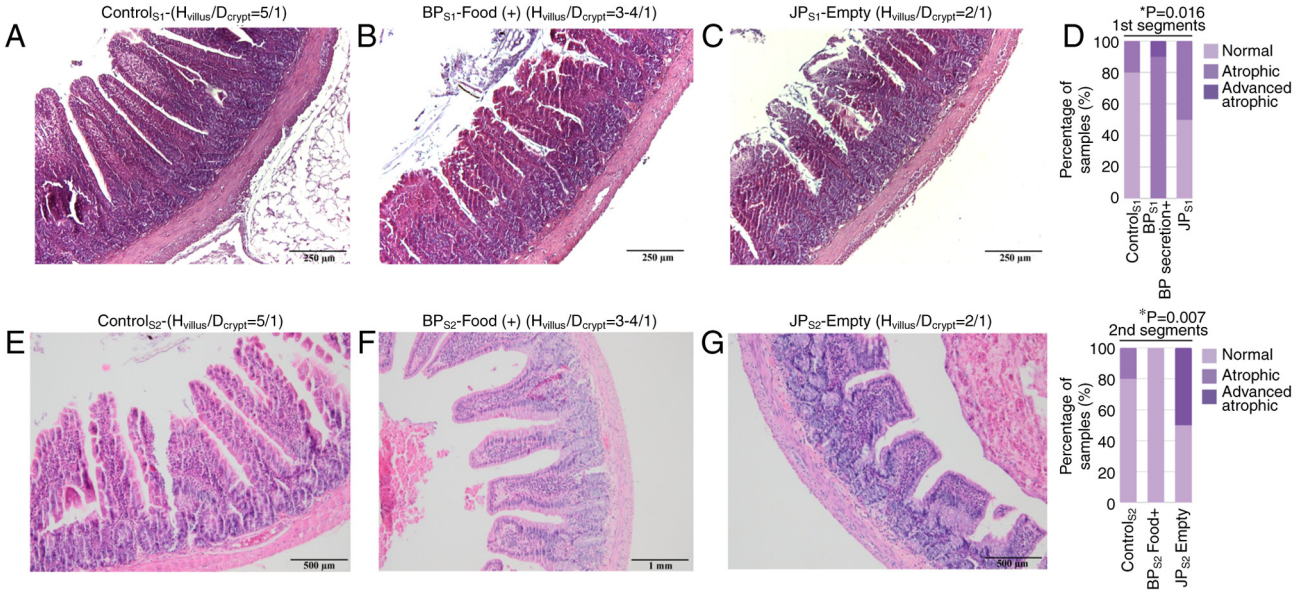

Figure 4. Representative microscopic images of villus/crypt ratios in different intestinal segments. S1 segments: (A) Normal mucosa (villus/crypt ratio ~5:1). (B) Atrophic mucosa (moderate atrophy; villus/crypt ratio ~3-4:1). (C) Advanced atrophic mucosa (severe atrophy; villus/crypt ratio ~2:1). (D) Villus height/crypt depth ratios across the experimental groups. The most pronounced atrophy was observed in the JPS2 segment, which lacked both food and bile (P=0.007). S2 segments: (E) Normal mucosa (villus/crypt ratio ~5:1). (F) Atrophic mucosa (moderate atrophy; villus/crypt ratio ~3-4:1). (G) Advanced atrophic mucosa (severe atrophy; villus/crypt ratio ~2:1).

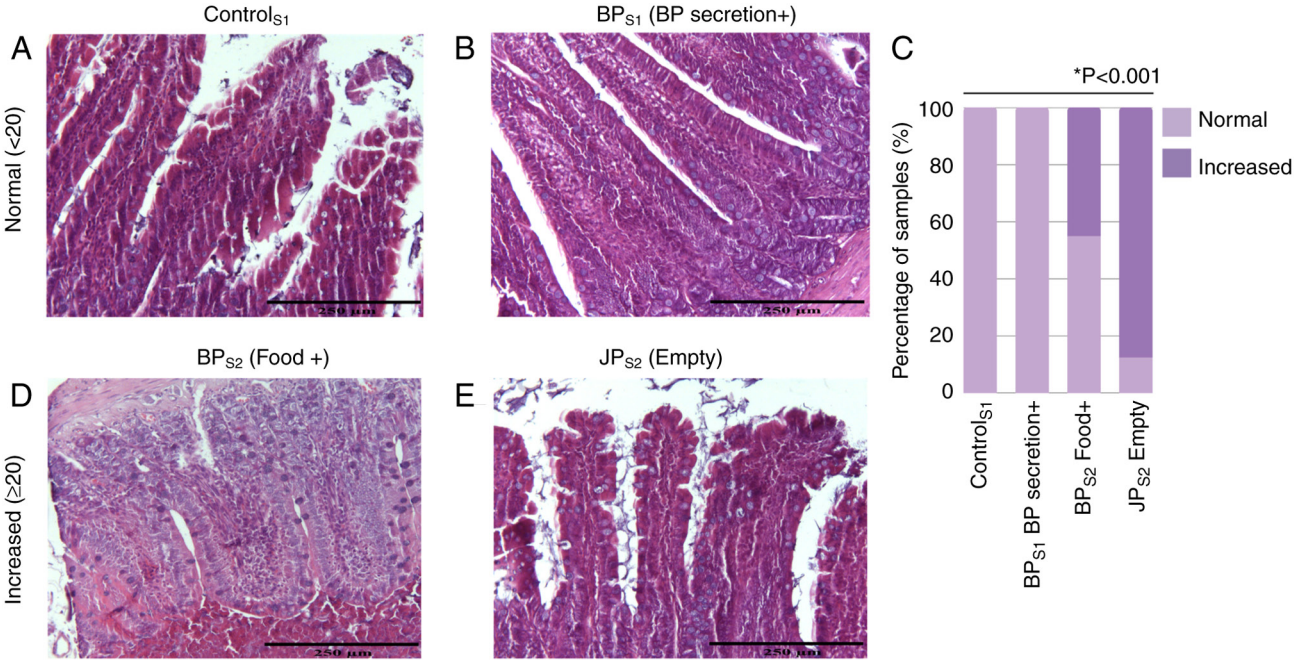

Figure 5. Representative microscopic images and quantification of intraepithelial lymphocytes (IELs) in the compared intestinal segments. (A) Control S1 segment (normal passage of both food and biliopancreatic secretions). (B) BPS1 segment (passage of biliopancreatic secretions only). (C) Quantification of IEL counts among the four segments. (D) BPS2 segment (passage of food only). (E) JPS2 segment (no passage of either food or biliopancreatic secretions). A significant increase in IEL counts was observed compared with Control S1 (*P<0.001).

all groups were compared, the most severe atrophy occurred in JP$_{S2}$ (no food or bile; Fig. 4D). No significant differences were found in the third segments (common limb; S3).

IEL count. IEL counts were assessed based on the number of intraepithelial lymphocytes per 100 epithelial cells as described (Fig. 5A, B, D-E). IEL counts were compared across segments with differing exposure to luminal contents: Control S1 (food + biliopancreatic secretions), BPS1 (biliopancreatic secretions only), BPS2 (food only), and JPS2 (neither food nor biliopancreatic secretions). IEL counts were significantly higher in the segments lacking biliopancreatic secretions (BPS2 and JPS2), with the highest counts observed in JPS2 (Fig. 5C). No significant differences were observed in the S3

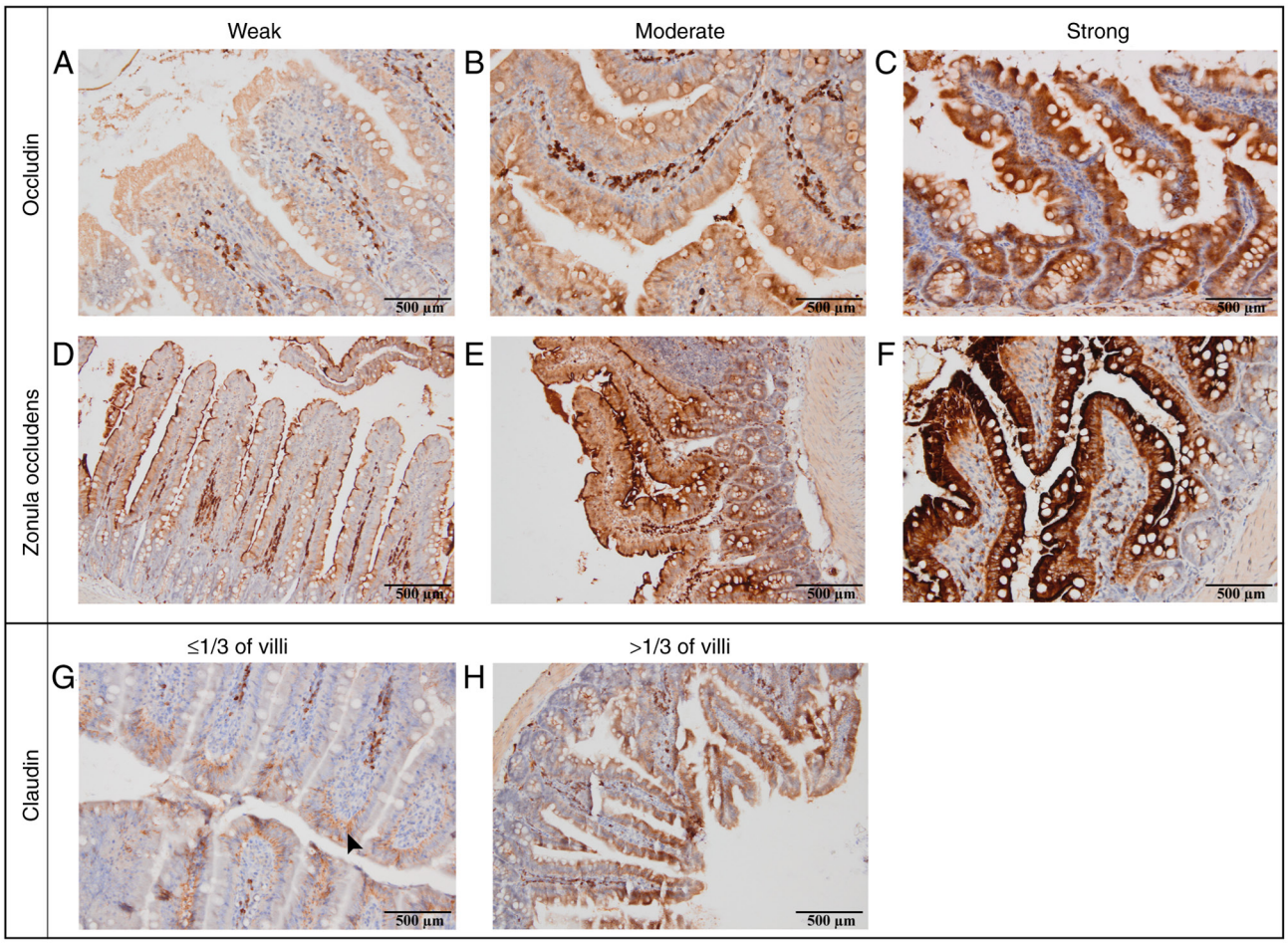

Figure 6. Representative immunohistochemical staining patterns of tight junction proteins in different intestinal segments. Occludin staining: (A) Weak occludin expression. (B) Moderate occludin expression. (C) Strong occludin expression in the BPS2 segment. (D-F) ZO-1 staining: (D) Weak ZO-1 expression in the JPS2 segment. (E) Moderate ZO-1 expression in the BPS1 segment. (F) Strong ZO-1 expression in the BPS2 segment. Claudin-1 staining patterns in villus epithelium: (G) Claudin-1 expression extending to more than one-third of villus height. (H) Claudin-1 expression limited to the lower one-third of villus height. These representative images demonstrate the segment-dependent variation in tight junction protein expression associated with the presence or absence of luminal food and biliopancreatic secretions. BP, biliopancreatic diversion; JP, jejunal bypass; S, segment; ZO, zonula occludens.

(common limb) segments when comparing the Control, BP, and JP groups, as this region receives both luminal nutrients and biliopancreatic secretions in all models.

TJ protein expression. The staining intensity and distribution patterns of occludin, ZO-3, and claudin-1 were evaluated according to the semi-quantitative criteria shown in Fig. 6A-H (weak, moderate or strong staining for occludin and ZO-3; and ≤1/3 vs. >1/3 villus height for claudin-1). No statistically significant changes in ZO-3 expression were observed across any segment. The results demonstrated slight variations in BP_{S1} and BP_{S2}, but this did not reach statistical significance.

For claudin-1, when the first segments (S1) across all groups were compared, no significant differences in claudin-1 staining patterns were observed. Expression levels of claudin-1 were significantly reduced in intestinal JP_{S2} segment when compared with expression in the control group (Fig. 7A). No differences were noted in the third segments (S3). In the S1 segment, occludin staining intensity was significantly higher in the JP group compared with the Control and BP groups. By contrast, no significant differences were observed among the groups in the S2 and S3 segments. Compared with the control segment (S1, where both food and biliopancreatic secretions were present), occludin staining intensity was slightly increased in the BPS1 segment (bile only) and the JPS2 segment (neither food nor bile). The highest occludin expression was observed in the BPS2 segment, where only food was present (Fig. 7B).

Jejunal aerobic bacteria. Compared with control group, bacteria population in related segments on values of log10(CFU/mg) had no noticeable change in segment where only bile passed, an increase in segment where only food passed, a decrease in segment which included neither food nor bile (Fig 8). The difference among them was recorded statistically significant.

Plasma LPS and citrulline. Plasma LPS and citrulline levels represent one measurement per animal obtained from portal blood and are reported as group mean values. Plasma LPS levels did not differ significantly between groups. Plasma citrulline levels were highest in controls (8±2.0 nmol/ml) and lowest in JP group segments (6±1.7 nmol/ml), but this difference was not significant (Fig. S1C).

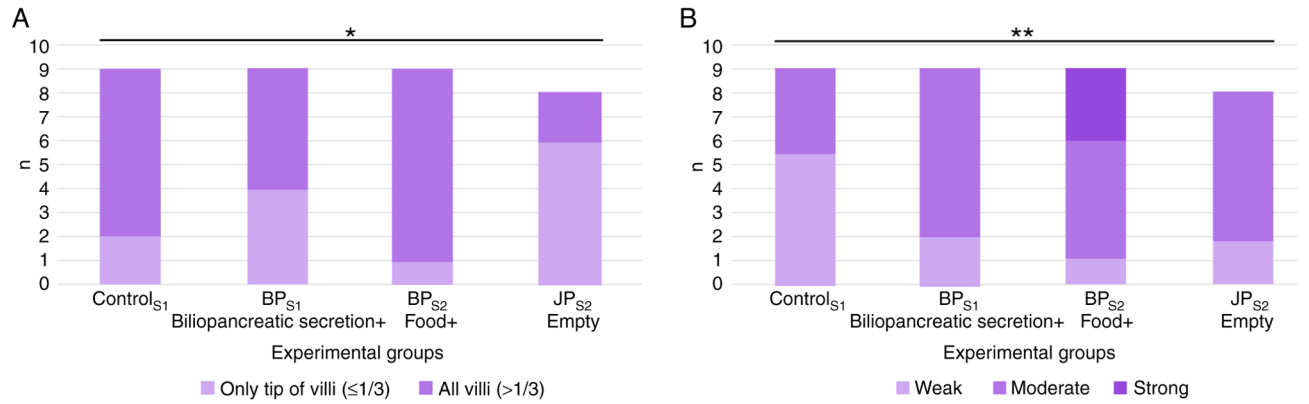

Figure 7. Expression of tight junction proteins in intestinal segments following surgical intervention. (A) Quantitative graph showing claudin-1 expression patterns, with a significant reduction in the JPS2 segment compared with the Control S1 segment (*P=0.035). (B) Quantitative graph showing occludin staining intensity across groups. A statistically significant difference was observed (**P=0.016). BP, biliopancreatic diversion; JP, jejunal bypass; S, segment.

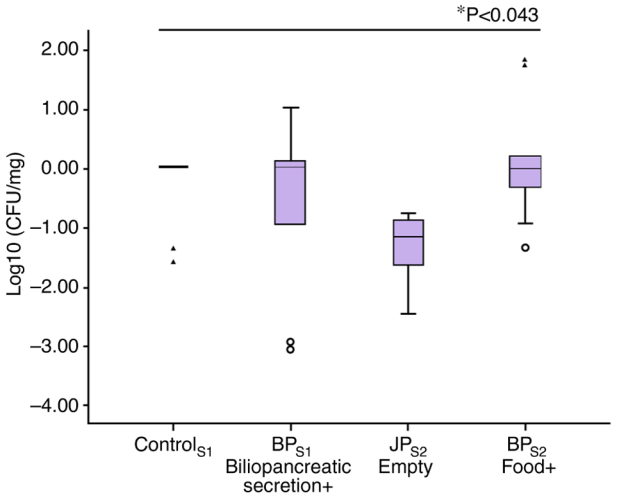

Figure 8. Graphic representation of bacterial jejunal aerobic population. *P=0.043. CFU, colony-forming unit; BP, biliopancreatic diversion; JP, jejunal bypass; S, segment.

Discussion

The largest microorganism reserve in the human body is found in the gastrointestinal tract (10). The physical, chemical and immune barrier functions of the gastrointestinal system prevent bacteria from spreading and invading the systemic circulation (11). TJ proteins between intestinal cells serve a major role in the regulation of intestinal permeability; notably, altered intestine permeability is associated with endotoxemia and may be involved in the pathogenesis of a number of diseases, including inflammatory bowel disease, celiac disease, type 2 diabetes, obesity, and non-alcoholic fatty liver disease (12). The effects of enteral nutrition on intestinal integrity have been widely studied; the literature consistently highlights enteral nutrition in maintaining intestinal structure and function due to its physiological engagement of the gut (13,14). However, to the best of our knowledge, there are limited studies on the individual effects of bile and pancreatic secretion or food.

In the present study an experimental rat model was used, jejunum segments were surgically created through which biliopancreatic secretions, food or both did not pass. The intestinal segments were long enough to prevent malnutrition as a confounding factor. Consequently, there were no significant changes in body weight among the groups throughout the experimental period.

The present study showed that occludin expression, which has a key regulatory role among TJ proteins, was significantly affected depending on the presence of food within the lumen. Specifically, the staining intensity of occludin was increased in the segment where only food passed (BPS2), indicating that direct luminal nutrient exposure was associated with enhanced occludin expression. The present finding is supported by another experimental study, which showed that occludin increases with enteral nutrition compared with parenteral nutrition (15). Oral nutrients are key in building up the gastrointestinal system from the beginning of life (16). The segment receiving only luminal nutrients (BPS2) demonstrated the strongest occludin staining, supporting the concept that direct exposure to dietary content helps preserve TJ integrity throughout the lifespan. A decrease in the expression of TJ proteins is associated with necrotizing enterocolitis in the neonatal period, highlighting the importance of proper nutrition in the intestines (17).

Claudin-1 is a TJ protein found in numerous tissues besides the intestine, including the epidermal TJs in skin, the bile canalicular membrane in the liver, and the distal renal tubules in the kidney. These distributions highlight its broader role in maintaining epithelial barrier function across organ systems (18,19). Its expression has been shown to be decreased in enterocytes in a number of conditions including allergies, pancreatitis, cholangitis, colon cancer and inflammatory bowel disease (20). In the present study, claudin-1 expression was decreased in the absence of food, and the decrease was more pronounced in the absence of both biliopancreatic secretions and food. However, no change was observed in the absence of biliopancreatic secretions alone. Identifying the components of food that are most important for improving the condition of TJ proteins will require further studies (21).

In the present study, the expression of ZO-3 was examined; ZO-3 is a member of the ZO protein family, which functions as a cytoplasmic scaffold linking transmembrane TJ proteins to the actin cytoskeleton (22,23). No statistically significant differences in ZO-3 expression among the experimental groups

were observed, suggesting that this structural protein may remain relatively stable in response to luminal changes such as the absence of food or biliopancreatic secretions. Similarly, in a high-fat diet mouse model by Murakami *et al* (15) ZO-1 expression remained unchanged, and no alterations were reported in the small intestine, supporting the idea that ZO proteins are more resistant to such changes. Collectively, these findings support the notion that members of the ZO protein family, particularly ZO-1 and ZO-3, may exhibit greater resistance to environmental or dietary perturbations compared with transmembrane TJ components.

The intestinal mucosa can adapt in response to different stimuli; this adaptation is key for survival in different conditions including short bowel syndrome and after bariatric surgery (24). In the present study, it was observed that after the surgical procedures, the animals in the experimental groups lost weight for 4-5 days and regained weight after this period. Therefore, preserving the weight of the animals and avoiding malnutrition are strengths of the present study. In the study by Taqi *et al* (25), weight loss and reduced mucosal growth were observed, particularly in segments deprived of luminal nutrients. In contrast, in our study, animal body weights were preserved throughout the experimental period, preventing malnutrition and reducing its potential confounding effects. In addition, citrulline levels, which were used as an indicator of functional enterocyte mass, did not differ between the groups. This suggests that functional enterocyte mass was preserved across the groups, helping to prevent malnutrition and its systemic effects. Although segment-specific structural and TJ protein differences were detected, the overall functional integrity of the intestine was maintained across groups. The intestinal segments through which food passed demonstrated gross enlargement (increased segment diameter and wall thickness). This macroscopic change may represent an adaptive response, helping to compensate for the bypassed segments that receive reduced luminal stimulation.

Mucosal integrity and growth are mediated by hormonal, neural, immune and mechanical signals (26). A reduction in mucosal integrity and barrier function is recognized as an early step preceding villous atrophy, as the breakdown of TJs increases permeability and compromises epithelial turnover. Mucosal atrophy is characterized by morphological changes of villus height, crypt depth, surface area and the number of epithelial cells (27). In the present study, it was shown that there was notable atrophy in the segments through which biliopancreatic secretions and food did not pass. The atrophy was less notable if either food or biliopancreatic secretions were present. The findings of the present study showed that food may induce enhanced adaptation compared with biliopancreatic secretions in terms of morphological features.

The interaction between the intestinal microflora and the host has been a subject of research in numerous studies (28,29). The current study presented that intestinal bacterial content significantly decreased in the segments through which food and biliopancreatic secretions did not pass. On the other hand, there was an increasing trend in the bacterial populations of the intestinal segments that had food but not biliopancreatic secretions [BPS2-food(+)] compared within the four-segment (Control S1, BP S1, BP S2, and JP S2). The effects of food and different diets on intestinal microbiota have been intensively explored in other studies and it has been shown that diet is one of the major factors affecting the microbiota (30,31).

The present study has some limitations: i) The effects of food and biliopancreatic secretions on intestinal morphology were examined; however, the individual effects of bile and pancreatic secretions were not determined; and ii) the focus was only on the aerobic population and other bacterial subgroups were not studied. Therefore future research should explore these areas further.

In conclusion, the present study demonstrated that biliopancreatic secretions and food regulate intestinal morphology, IEL count and the levels of TJ proteins, including occludin and claudin-1. Similarly, the intestinal microbiota was shown to be affected by food and gastrointestinal secretions. The present findings indicated that food has a more prominent role than gastrointestinal secretions in maintaining the morphology and integrity of the gastrointestinal tract.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

OC performed the investigation, analyzed data and wrote the original draft of the manuscript. AH performed experiments, data interpretation and formal analysis, and assisted in manuscript drafting and revision. BŞ and FA performed experiments. CS analyzed and interpreted data. OA conceived and designed the study, supervised the research process, interpreted data, and critically revised the manuscript for important intellectual content. All authors have read and approved the final manuscript. OC and AH confirm the authenticity of all the raw data.

Ethics approval and consent to participate

The present study was approved by the Animal Ethics Committee of Hacettepe University (approval no. 15/54-03; Ankara, Turkey).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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