Obesity Surgery (2019) 29:369–375
https://doi.org/10.1007/s11695-018-3540-1

ORIGINAL CONTRIBUTIONS

Gut Microbiota Imbalance Can Be Associated with Non-malabsorptive Small Bowel Shortening Regardless of Blind Loop

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Published online: 6 October 2018

Abstract

Introduction Some traditional bariatric surgery procedures may lead to functional gut shortening, which may unsettle the fine-tuned gastrointestinal physiology and affect gut microbiota balance.

Purpose Evaluate the gut microbiota behavior in rat models facing gut shortening due to intestinal bypass.

Materials and Methods Wistar rats ($n = 17$) were randomly distributed in three groups: (1) sham group ($n = 5$); (2) blind loop group ($n = 6$); and (3) resection group ($n = 6$). Intestinal samples and feces were analyzed to measure bacterial concentrations (small intestinal bacterial overgrowth—SIBO) 12 weeks after the experimental procedures. Bacterial translocation (BT) was investigated in the mesenteric lymph node (MLN), liver, spleen, and lung of the animals. In addition, inflammatory aspects were investigated in their liver and small bowel through histological analysis.

Results Regardless of blind loop, gut shortening groups recorded similar high level of bacterial concentrations in intestine compartments, greater than that of the sham group ($p \leq 0.05$). BT was only observed in the MLN of gut shortening models, with higher percentage in the blind loop group ($p \leq 0.05$). The gut and liver histopathological analysis showed similar low-grade chronic inflammation in both gut shortening groups, likely associated with SIBO/BT events.

Conclusion Sustained SIBO/BT was associated with proximal gut shortening in half regardless of blind loop, whereas the GI tract's ability to restore gut microbiota balance after a surgical challenge on the small bowel appears to be linked to the functional remaining gut.

Keywords Microbiota · Intestinal bypass · Blind loop syndrome · Rats

Introduction/Purpose

Morbid obesity is a chronic and multifactorial disease whose incidence increases worldwide, affecting patients' quality of life and survival mainly due to obesity-related comorbidities [1]. Bariatric surgery has emerged as an effective treatment in the last decades, since it enables significant long-lasting weight loss and comorbidities control. This outcome may explain the exponential increase in the number of procedures performed around the world [2, 3].

Although laparoscopic sleeve gastrectomy indications are currently at the top list of bariatric surgeons and their number keeps on growing [2, 4], many bariatric procedures involving functional gut shortening at varying degrees remain in place, such as the traditional Roux-en-Y gastric bypass (RYGB) [5]. Although RYGB was designed based on a short biliopancreatic limb and on an alimentary limb only to prevent bile reflux to the gastric pouch [6], bariatric surgeons currently have made the option for longer limbs, mainly when the simultaneous treatment of metabolic comorbidities such as type 2 diabetes mellitus (T2DM) is taken into consideration [7, 8].

Although gut shortening is intended for therapeutic purposes, its inclusion in some bariatric techniques may affect the physiology of the gastrointestinal (GI) tract, such as the well-coordinated signaling for gut microbiota balance. Several studies have already shown that bariatric procedures with intestinal bypass, such as RYGB, may cause long-term changes

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in gut microbial composition and diversity [9–13], whose overall implications remain under debate.

In addition, bariatric procedures may also lead to microbiota disarray, since they provide a "blind loop-like" small bowel segment. This outcome draws the attention to an old surgical paradigm that remains in surgical community. Based on this paradigm, an excluded bowel may work as a bacterial reservoir and may lead to small intestinal bacterial overgrowth (SIBO) and, subsequently, to bacterial translocation (BT).

Considering that the GI tract holds a complex microbiome, and that it plays multiple roles other than digestive and absorptive functions [14–16], it is possible stating that non-malabsorptive gut shortening may affect the physiological regulation of the GI tract and impact on gut microbiota balance, which, in its turn, may imply in several pathological conditions.

Thus, the aim of this study was to evaluate the gut microbiota behavior in rats that underwent non-malabsorptive surgical gut shortening with and without blind loop.

Materials and Methods

Seventeen adult female Wistar-EPM-1 rats were kept in individual cages under adequate environmental conditions, at 12-h day-night cycles. The animals fed on standard diet formulation (Labina, Nestlé Purina PetCare Company, Evialis International) and had access to water ad libitum. All applicable Institutional and National Guidelines for the Care and Use of Animals were followed, and the experimental protocol was approved by the Local Ethics Committee.

After body weight measurement, general anesthesia, and midline laparotomy to properly expose the small bowel, the animals were randomly assigned in three groups, based on the procedure performed through the microsurgery technique. In the sham group (SHG) ($n = 5$), a complete small bowel section was performed right after the duodeno-jejunal transition, followed by end-to-side unabsorbable one-layer enteroenteric anastomosis. In the remaining animals, a corresponding point to 50% of the overall small bowel length was identified. In the blind loop group (BLG) ($n = 6$), the proximal half of the small bowel was kept on site but excluded from the alimentary transit, whereas in the resection group (RG) ($n = 6$), the gut shortening was performed by withdrawing the proximal half of the small bowel. The alimentary transit was restored through end-to-side enteroenteric anastomosis in both groups, similar to the SHG (Fig. 1).

The small bowel was gently placed back into the abdominal cavity, which was closed through one-layer running suture. The animals returned to individual cages for 12-week postoperative follow-up—this monitoring period was defined in a previous pilot study about fecal bacterial growth kinetics.

After stool collection, the animals underwent general anesthesia and wide midline laparotomy to enable tissue sample extraction through aseptic technique in order to determine the aerobe and anaerobe facultative Gram-negative bacterial concentrations at the 12th week. Tissue samples were collected from the duodenum, pre- and post-anastomosis segments, ileum, and cecum of all groups for SIBO analysis. Samples were also collected from three blind loop portions in BLG: stump, middle, and pre-anastomosis segment (Fig. 2). Finally, BT incidence in the mesenteric lymph node (MLN), as well as and in liver, spleen, and lung fragments, was investigated by microbiological culture.

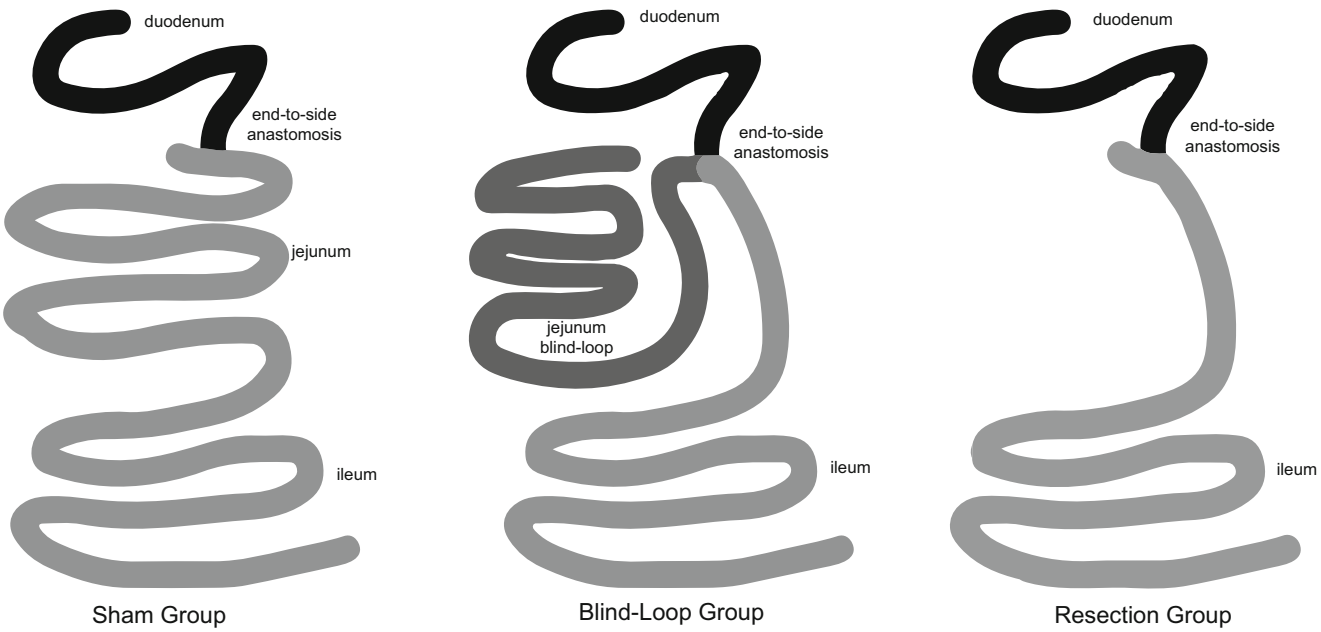

Fig. 1 Schematic drawing of the surgical procedure applied to all groups

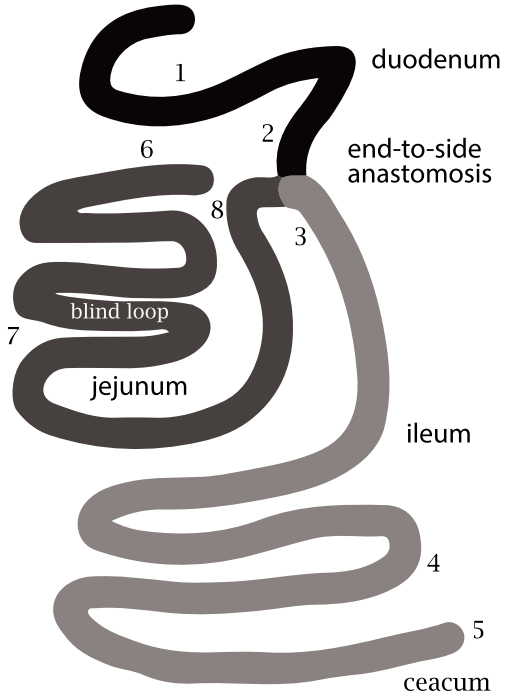

Fig. 2 Schematic drawing showing the sites where samples were collected for bacterial overgrowth analysis. (1) duodenum; (2) pre-anastomosis; (3) post-anastomosis; (4) ileum; (5) cecum; (6) blind loop stump; (7) blind loop middle; (8) blind-loop anastomosis

For the accomplishment of microbiological culture, a well-standardized technique in our laboratory was followed. Collected feces were weighed, diluted in 5 mL sterile saline, homogenized, and filtered for microbiological culture. Gut tissue samples, MLN, and liver, spleen, and lung fragments were weighted, macerated, diluted in 2 mL sterile saline, and filtered. A 100-mL aliquot of each sample was seeded on a culture plate containing MacConkey agar (Difco™ BD - Becton, Dickinson and Company, NJ, USA) and incubated at 37 °C, for 18–24 h, for subsequent colony counts. The bacterial recovery, a microbiological expression which means how amount of bacteria can be counted after a seeding in culture plate, was expressed as colony-forming units (CFU) per gram of tissue or feces and converted into logarithm base.

In addition, histological analysis was applied to the liver, to pre- and post-anastomotic intestinal segment, and to ileum samples. Fragments were immersed in 4% formaldehyde solution, embedded in hystoresin blocks, cut in 2-μm slices, stained with hematoxylin and eosin (HE), and examined under light microscopy. Morphological analysis aimed at describing inflammatory patterns, whenever applicable ($n = 3$/group).

Statistical Analysis

Given the already expected lack of standard normal data distribution, the statistical analysis was performed through nonparametric tests, and descriptive analyses were expressed in boxplots. Nonparametric ANOVA was used to analyze two concomitant factors: one uncorrelated (GROUPS) and one correlated (or TIME-BODY - "compartment"). Kruskal-Wallis test was used to compare variables recorded for three independent groups (SHG vs. BLG vs. RG). Mann-Whitney and Wilcoxon tests for paired samples were adopted in case of statistically significant differences among the three groups. Wilcoxon paired test was adopted to compare the weights of animals within each group (2 related samples—"before and after"—"preoperative vs. postoperative"). Friedman's test was used to compare segments within each group, whereas Fischer's exact test was used to analyze categorical variables. Data were compiled into spread sheets (Excel 2010, Microsoft Office System®) to EXSTAT complement and a p value ≤ 0.05 was set as significance level.

Results

The periodic monitoring of fecal kinetics showed high bacterial recovery rate in all groups as early as the second postoperative day on concentrations that ranged from 10^7 to 10^9 CFU/g. This fecal SIBO status remained unchanged until the fourth week in SHG and gradually returned to the basal concentration (10^4 to 10^6 CFU/g) until the sixth week. On the other hand, rats subjected to gut shortening kept fecal bacterial overgrowth until the end of the study (12th week). Additional analyses conducted in our laboratory showed sustained bacterial overgrowth for at least 80 weeks (data not shown).

All animals gained weight throughout the study, regardless of gut shortening and fecal SIBO status. Weight gain was slightly higher in SHG (16%) than in RG (6%) and BLG (4.5%); however, comparisons between, and within, groups (before vs. after the procedure) did not show statistical significance (NS).

Regarding SIBO analysis, it was not limited to neighboring segments of the enteroenteric anastomosis, but it affected all other intestinal compartments and the stools. Both gut shortening groups showed higher bacterial concentration than the SHG in all intestinal segments and stools ($p \leq 0.05$), regardless of blind loop. On the other hand, the comparison between BLG and RG groups was similar to each other (Fig. 3).

The bacterial recovery within the blind loop showed negative culture in all stump samples. Notwithstanding, there was bacterial recovery in the middle portion and in higher level proportion close to the anastomosis (10^7–10^8 CFU/g). This finding featured SIBO status similar to that of the pre- and post-anastomosis gut segments (Fig. 4).

BLG recorded higher BT positivity in MLN (83%) than the RG (50%) ($p \leq 0.05$), whereas SHG did not show BT, as expected for a group with no SIBO (Fig. 5). Likewise, no

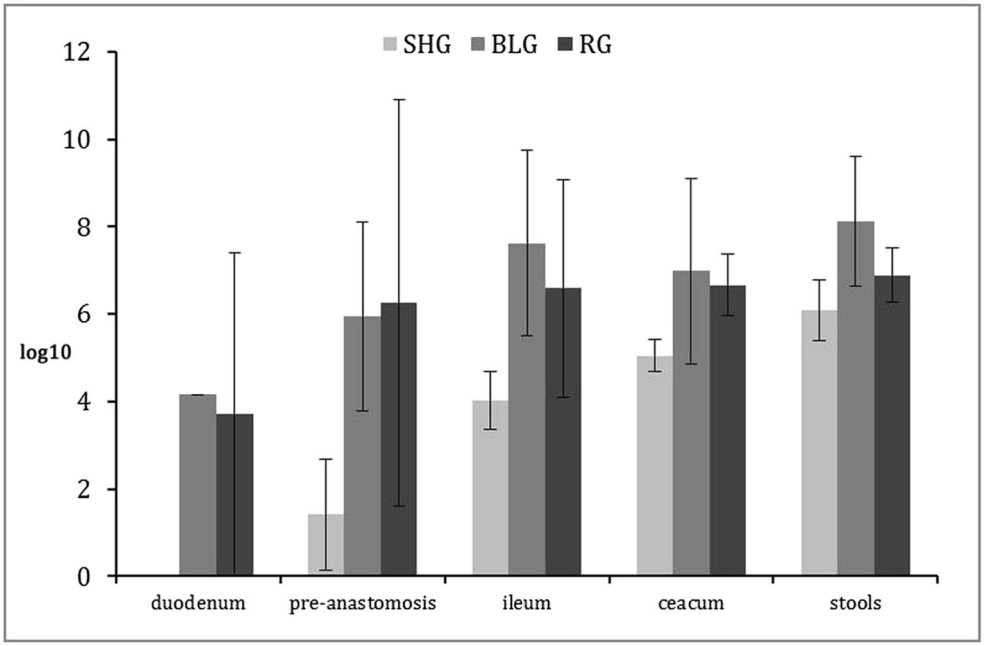

Fig. 3 Comparison between bacterial concentrations in varying GI compartments and stools. Data expressed as CFU/g of tissue or stools in $\log_{10}$. SHG, sham group; BLG, blind loop group; RG, resection group. Nonparametric ANOVA between groups: $p \leq 0.05$. Nonparametric ANOVA between organs: $p \leq 0.05$. Graphic data expressed in columns with mean ± SD

bacterial translocation to the lung, spleen, and liver was observed in any of the groups.

Histological analysis showed discrete and sparse gut inflammation in SHG, while liver fragments were normal. Both gut shortening groups showed intense lymphocytic inflammatory infiltration in the small bowel villi associated with morphological patterns such as flattening, enlargement, and apical necrosis. Furthermore, liver fragments of both gut shortening groups showed intense lymphocyte infiltration at the portal space, besides cellular edema, sinusoidal vascular congestion, and cytoplasmic microvesicles, which is indicative of micro steatosis (Fig. 6).

Discussion

The historical and empirical safety limit of gut shortening procedures has been mainly based on absorptive physiology maintenance. Accordingly, surgical gut shortening in half, either through exclusion or resection, is often seen as a safe procedure given the usual lack of threatening clinical malabsorption features. However, this concept should be strongly mistrusted if one takes into consideration the gut microbiota balance, since our findings showed long-lasting SIBO with BT events, regardless of blind loop.

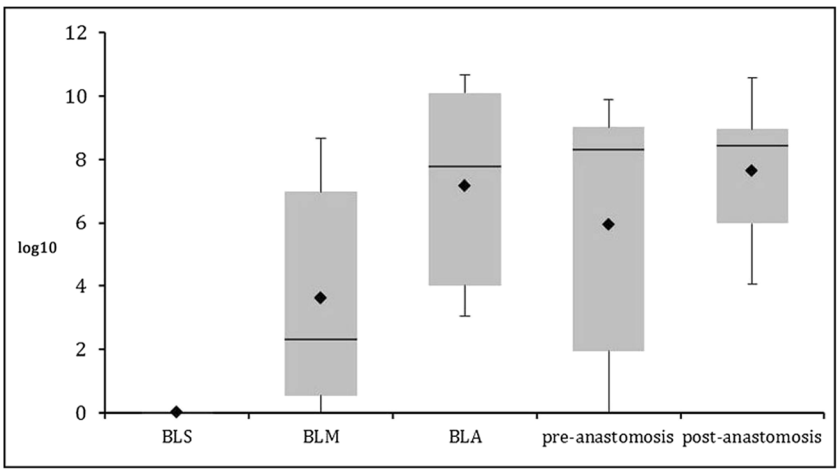

Fig. 4 Comparison between bacterial concentrations in the blind loop and in segments close to the anastomosis (pre- and post-anastomosis). Data expressed as CFU/g of tissue in $\log_{10}$. BLS, blind loop stump; BLM, blind loop middle; BLA, blind loop anastomosis. Friedman, all segments—$p \leq 0.05$; Friedman, BLS × BLM × BLA—$p \leq 0.05$; paired sample Wilcoxon, BLS × BLM—$p = 0.06$ (NS); paired sample Wilcoxon, BLM × BLA—$p \leq 0.05$; paired sample Wilcoxon, BLS × BLA—$p \leq 0.05$; paired sample Wilcoxon, BLA × pre-anastomosis—NS; paired sample Wilcoxon, BLA × post-anastomosis—NS

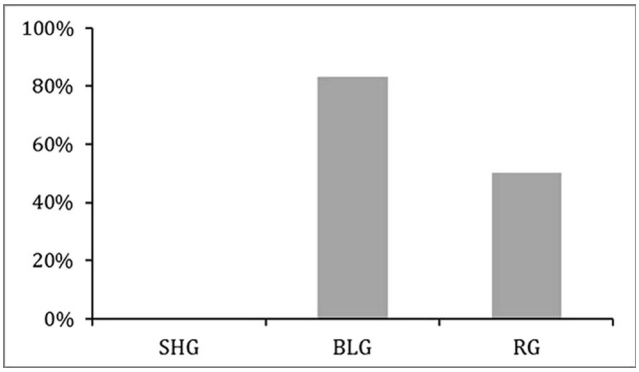

Fig. 5 BT positivity rate in MLN. SHG, sham group; BLG, blind loop group; RG, resection group. Fischer test, SHG vs. BLG—$p \leq 0.05$; SHG vs. RG—NS; BLG vs. RG—NS

This study also highlighted that weight monitoring should not be seen as a reliable parameter to predict GI dysfunction in animals subjected to gut shortening, unless in severe cases such as the short bowel syndrome, since the herein experimental models subjected to gut shortening did not show enough clinical malabsorption features and all animals with SIBO status presented weight gain throughout the postoperative period.

Despite the use of microsurgical technique, the SIBO process may have been triggered by the general surgical trauma associated with small bowel transection and with hand-sewn enteroenteric anastomosis, since there was bacterial overgrowth in the fecal kinetics of all groups. However, unlike observations in both gut shortening groups, such overgrowth was transient in SHG and lasted up to 6 weeks. This outcome allows stating that the preservation of the full small bowel length in the feed stream was a pivotal condition for gut microbiota rebalancing.

After the sixth postoperative week, and based on the SHG behavior, it was clear that the gut shortening in half was able to break down the finely tuned GI regulation system and led to dysfunctional GI tract, which hindered gut microbiota balance recovery. The bacterial concentration analysis applied to several gut compartments at the end of the study confirmed this besides showing that SIBO was not limited to the surgical site. It happened throughout the digestive tube and reached compartments far away from the enteroenteric anastomosis.

Moreover, the spontaneous SIBO found in both gut shortening groups exceeded any expectation, since it recorded up to 10^{10} CFU/g. As the regular bacterial concentration in the proximal small bowel ranges 10^2–10^4 CFU/g, and since SIBO diagnosis can be set at 10^5 CFU/mL [17], the amount of bacterial overgrowth observed in the current study was relevant because concentrations higher than 10^7 CFU/g have already proved to be a major BT factor, even in healthy guts [18, 19].

Although the exceedingly high SIBO levels in the intestinal segments and stools were similar in both gut shortening groups, the highest MLN positivity rate was recorded for BLG. This outcome may be associated with greater gut surface presenting SIBO in BLG, since the "blind loop anastomosis" segment was also affected by bacterial overgrowth. Furthermore, a plausible change in the food flow dynamics near the anastomosis, with luminal "stagnation", could increase the time of intestinal mucosa was exposed to the microbiota in SIBO status and favor bacterial translocation. However, further studies are necessary to investigate both "area-factor" and "time-factor" in BT events.

SIBO status in the "blind loop anastomosis" segment may also suggest the existence of a microbial reservoir within the

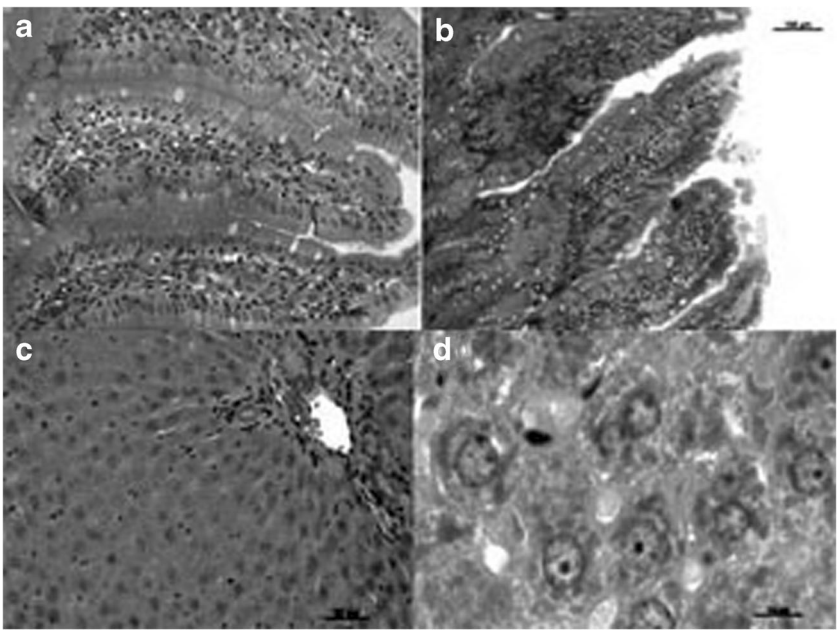

Fig. 6 Demonstrative histological findings in the small bowel and liver. **a** Typical normal aspect of small bowel villi in the sham group. **b** Increased cell infiltration and villi tip loss characterizing histological changes often seen in gut shortening groups. Liver changes in gut shortening groups showing lymphocytes infiltration around the centrolobular vein (**c**) and microsteatosis in hepatocytes (**d**)

blind loop. However, if one takes into account all blind loop segments, the bacterial concentration decreased from the anastomosis to the stump, a fact that allowed us to consider that the SIBO status within the blind loop was a condition related to the overgrowth observed in the nearest intestinal segments (pre- and post-anastomosis). In addition, similar bacterial recovery in several segments of both gut shortening models clearly indicates that gut shortening itself, and not the blind loop, was the major factor for SIBO. Some previous studies based on a short-bypassed gut segment are at odds with our findings, corroborating the old paradigm of blind loop as bacterial reservoir [20]. Although it is still in force, these old paradigms had been already contradicted by studies based on longer blind loops [21, 22], and now by our findings.

Based on the histological analysis, aspects such as chronic inflammation with morphological villi disarray were closely related to the bacterial overgrowth found in the intestinal segments of both gut shortening groups, suggesting mucosal barrier dysfunction, which may have contributed to BT. In addition, there was systemic inflammatory repercussion featured by significant liver damage consistent with a pattern of non-alcoholic steatohepatitis (NASH).

Although some correlation can be made between long-limb RYGB and biliopancreatic diversion with duodenal switch (BPD/DS), our study did not aim to mimic any bariatric procedures, but to study the effects of non-malabsorptive surgical rat model of gut shortening on microbiota balance. As clearly showed, the proximal gut shortening in half, either through exclusion or resection, was a crucial factor for the maintenance of the long-lasting SIBO, which was most likely triggered by surgical trauma and enteroenteric anastomosis. Further studies about the safe limit of gut shortening for microbiota balance should be conducted to guide technical drawings in gastrointestinal and bariatric surgeries.

In addition, BT events were related to the SIBO status and to morphological changes in the intestinal villi. Thus, studies about the global immunological and metabolic repercussion in face of this animal gut shortening model would be timely, since SIBO/BT may be involved with the source, maintenance, or worsening of a wide range of chronic diseases such as inflammatory bowel disease [23], NASH [24, 25], T2DM [26, 27], and with obesity itself [28, 29].

Therefore, proximal gut shortening in half may not be as harmless as it seems, since it was a major factor for the sustained GI physiology disruption featured by long-lasting microbiota disarray, regardless of blind loop.

Conclusion

Sustained SIBO/BT was associated with proximal gut shortening in half regardless of blind loop, whereas the GI tract's ability to restore gut microbiota balance after a surgical challenge on the small bowel appears to be linked to the functional remaining gut.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Ethical Approval All applicable institutional and national guidelines for the care and use of animals were followed. The present study was previously approved by Local Ethics Committee (UNIFESP – 0215/11).

Informed Consent Does not apply.

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