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BRIEF COMMUNICATION

Duodenal-Jejunal Bypass Maintains Gut Permeability by Suppressing Gut Inflammation

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Abstract

Background The aim of this study was to investigate gut inflammation and permeability in rats after duodenal-jejunal bypass (DJB) and in rats injected with a glucagon-like peptide-1 (GLP-1) receptor analog.

Methods Twelve male 16-week-old obese diabetic rats were divided into three groups: the DJB group, the sham group, and the group injected daily with a GLP-1 receptor agonist (liraglutide). Gut inflammation and the expression of tight junction protein (claudin-1) were analyzed in the three groups at 8 weeks after surgery.

Results The DJB group showed significantly lower levels of gut inflammatory cytokines than the liraglutide group. Claudin-1 showed stronger intensity on immunofluorescent staining in the DJB group than that in the liraglutide group.

Conclusions In summary, DJB surgery might maintain gut permeability via suppression of gut inflammation.

Keywords Gut inflammation · Gut permeability · Tight junction protein (claudin-1) · GLP-1 receptor agonist (liraglutide) · Gut inflammatory cytokines (IFN-γ, IL-1, IL-6, and TNF-α) · Roux-en-Y reconstruction · Bariatric surgery

Abbreviations

DJB	Duodenal-jejunal bypass
GLP-1	Glucagon-like peptide-1
HFD	High-fat diet
RNA	Ribonucleic acid
RT	Reverse transcription
PBS	Phosphate-buffered salts
DAPI	4′,6-Diamidino-2-phenylindole
LPS	Lipopolysaccharide
NASH	Nonalcoholic steatohepatitis

Introduction

Bariatric surgery has been reported to be the most effective procedure for the treatment of obesity and obesity-related diseases. This procedure can improve obesity and insulin resistance and decrease the risk of cardiovascular disease [1]. In 2017, 95,125 patients in 18 Asia-Pacific countries underwent bariatric/metabolic surgery, with 68.0% undergoing sleeve gastrectomy and 19.5% undergoing bypass surgery [2].

A duodenal-jejunal bypass (DJB) is a metabolic procedure involving duodenal and proximal jejunal exclusion to nutrients, a jejunal Roux-en-Y reconstruction, and early nutrient delivery to the distal small bowel. Rubino et al. showed dramatic improvements in glucose homeostasis after DJB surgery [3].

In the authors' previous report, DJB improved diabetes and steatohepatitis through enhanced glucagon-like peptide-1 (GLP-1) secretion via increased bile acids and the proliferation of L cells in the ileum in comparison with a GLP-1 receptor agonist (liraglutide) injection. DJB surgery might be a key strategy for treating obese patients with type 2 diabetes [4]. Furthermore, the authors reported that DJB changed the composition of gut microbiota, and these changes might be the factors that contribute to the effects of DJB [5].

A high-fat diet (HFD) was found to increase intestinal inflammatory cytokines and compromise mucosal barrier integrity with a loss in the tight junction protein (claudin-1) and increase the severity of colitis, which leads to insulin resistance due to inflammation of the liver and adipose tissue [6]. Regarding inflammatory cytokines, tumor necrosis factor

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(TNF)-α, interleukin (IL)-1 and IL-6, and interferon-γ were closely associated with obesity and insulin resistance [7–10]. However, the effects of DJB on gut inflammation and permeability remained unclear.

This study investigated gut inflammation and permeability in rats after DJB surgery and in rats injected with a GLP-1 receptor analog.

Materials and Methods

Animals

Twelve male 16-week-old Otsuka Long-Evans Tokushima Fatty (OLETF) rats (Japan SLC Inc., Hamamatsu, Japan) were used for this experiment. The rats were housed in a controlled environment. All experimental procedures were approved by the Division for Animal Research Resources, Institute of Health Biosciences, Tokushima University.

Methods

Duodenal-Jejunal Bypass, Sham Operation, and Liraglutide Injection

The 12 rats were randomly divided into three groups: the DJB group, the sham group, and the GLP-1 receptor agonist (liraglutide) injection group. There were four rats in each group. The rats were fasted overnight before the operations and were anesthetized with 2% to 3% isoflurane and air/oxygen for the operations. The DJB surgery was performed as reported by Rubino et al. [3]. That is, after a midline skin incision, DJB surgery was performed by postpyloric transection of the duodenum, closure of the duodenal stump, transection of the jejunum 10 cm from the ligament of Treitz, reconstruction of the intestinal passage by duodenojejunostomy, and finally connection of the biliopancreatic limb to the jejunum 15 cm distal from the duodenojejunostomy (Fig. 1). In the sham group, a midline laparotomy was performed, and the incision was then closed. This group showed the effect of an HFD on gut inflammation and tight junctions, compared with the other two groups. In the liraglutide group, a subcutaneous injection of 200 μg/kg/day liraglutide (Novo Nordisk, Bagsvaerd, Denmark) was administered every 24 h for 8 weeks. The rats in all groups were fed ad libitum a methionine- and choline-deficient HFD for 8 weeks postoperatively.

Ribonucleic Acid Isolation and Quantitative Real-time RT-PCR

Total ribonucleic acid (RNA) was extracted using the RNeasy Mini kit (Qiagen, Valencia, CA, USA) and reverse transcribed with the High Capacity cDNA Reverse Transcription kit (Applied Biosystems, Foster City, CA, USA). Quantitative real-time reverse transcription (RT)-PCR proceeded using a 7500 Real-Time PCR system with the TaqMan Gene Expression Assay-on-Demand and TaqMan Universal Master Mix (Applied Biosystems). Levels of gut inflammatory cytokines IFN-γ, IL-1, IL-6, and TNF-α (IFN-γ: Rn00594078_m1; IL-1: Rn00580432_m1; IL-6: Rn00561420_m1; and TNF-α: Rn00562055_m1) (Applied Biosystems) were assayed, and the control gene was TaqMan Rat GAPDH endogenous control (GAPDH; 4352338E; Applied Biosystems). The thermos-cycling conditions comprised 2 min at 50 °C, 10 min at 95 °C, 40 cycles of 15 s at 95 °C, and 1 min at 65 °C. Data were analyzed using the Applied Biosystems Prism 7500 Sequence Detection System version 1.3.1.

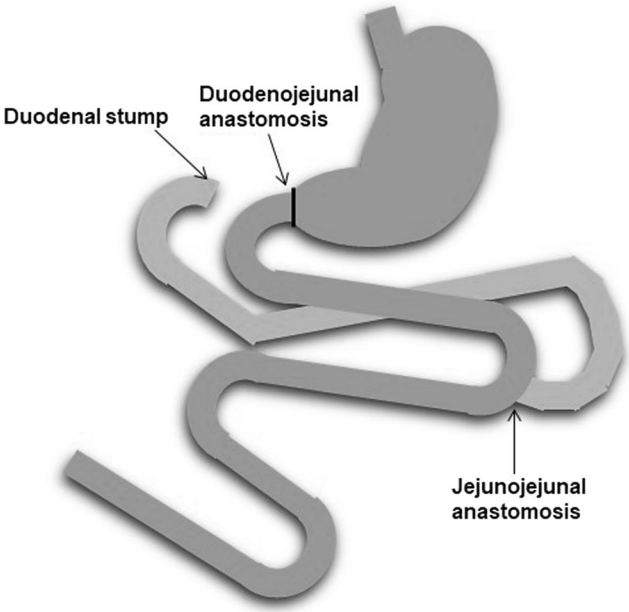

Fig. 1 Schematic drawing of the DJB surgery. The Roux limb is the alimentary limb from the duodenojejunal to the jejunojejunal anastomosis. The biliopancreatic limb is the limb carrying the biliopancreatic juices to the common limb. The common limb is the limb from the jejunojejunal anastomosis to the terminal ileum

Immunofluorescent Staining

Specimens (1 × 1 cm^2) of all layers in the common limb were harvested from the DJB group at sacrifice. Specimens (1 × 1 cm^2) of all layers in the distal small intestine and colon were harvested from the sham and liraglutide groups. All specimens were fixed in 10% formalin, embedded in paraffin, and cut into 4-μm-thick sections. For two-color immunofluorescent staining, the primary antibody for claudin-1 was detected with Alexa Fluor 555–conjugated goat anti-rabbit IgG (Invitrogen) (1:500 dilution) for 60 min, and DAPI was detected with sc-3598 (Santa Cruz) for 10 min. Finally, the slides were washed in PBS. Slides were then viewed and photographed under a confocal laser scanning microscope (KEYENCE, BZ-X700).

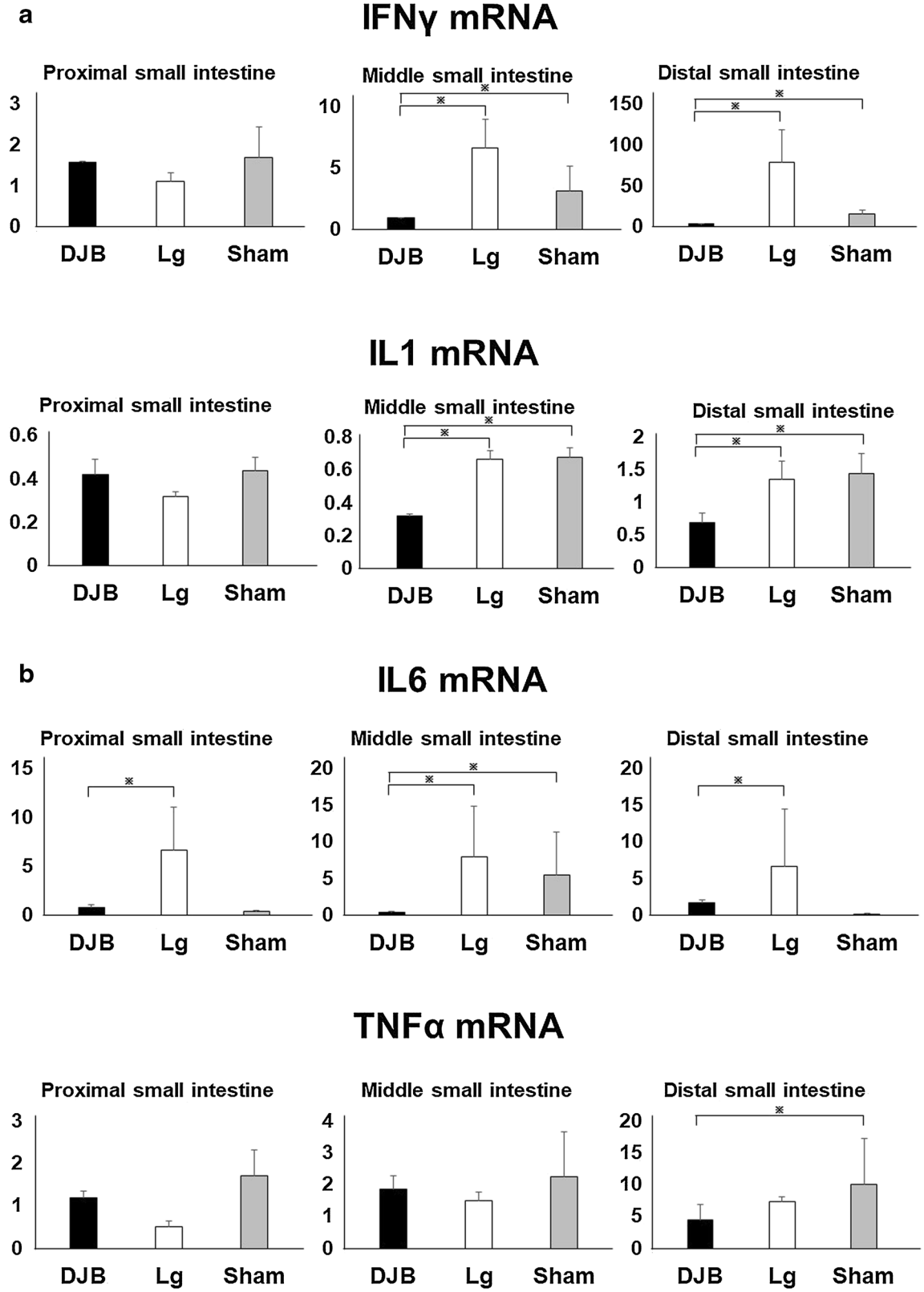

Fig. 2 **a**, **b** A comparison of the inflammatory cytokines in the small intestine of the DJB, liraglutide injection, and control groups. *$p < 0.05$. Values are expressed as means ± SEM (one-way ANOVA with Bonferroni's post hoc test)

The immunohistochemical findings were reviewed in a blinded fashion.

Statistical Analysis

Data were statistically analyzed using either unpaired Student's *t* test or one-way ANOVA with Bonferroni's post hoc test. Differences were considered significant at $p < 0.05$. The results are expressed as means ± standard error of the mean (SEM). All statistical data were generated using StatView version 5.0 for Window (SAS Institute Inc., Cary, NC, USA).

Results

The DJB group showed significantly lower levels of gut inflammatory cytokines (IFN-γ, IL-1, IL-6, and TNF-α mRNA) than the other two groups, especially in the distal small intestine (Fig. 2a, b). In addition, claudin-1 in the distal small intestine (ileum) of the DJB group showed strong intensity on immunofluorescent staining (Fig. 3). A high level of claudin-1 showed maintenance of gut permeability. Thus, DJB might suppress gut inflammation and maintain gut permeability.

Discussion

This study investigated the effects of DJB surgery on gut inflammation and permeability in obese diabetic rats and in rats injected with liraglutide.

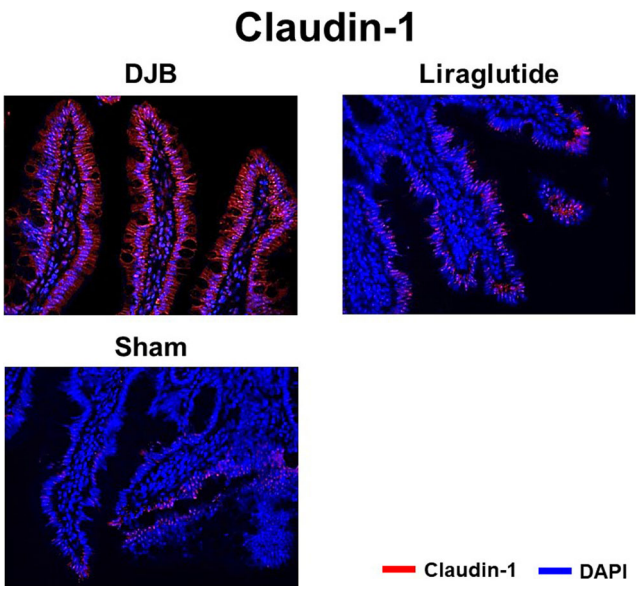

Fig. 3 Immunofluorescence staining of claudin-1 in the distal small intestine of the DJB, liraglutide injection, and control groups at postoperative week 8

The DJB group of rats showed low levels of IFN-γ, IL-1, IL-6, and TNF-α and a high level of claudin-1 expression in the ileum compared with the sham and liraglutide groups, which indicated that DJB might maintain gut permeability via suppression of gut inflammation.

In the authors' previous report, DJB surgery resulted in elevated bile acids and GLP-1, which are important in the modulation of metabolism and the improvement of insulin resistance [4].

It is well known that an HFD will induce chronic gut inflammation and insulin resistance. Yoshinaga et al. reported the correlation between gut inflammation and insulin resistance [11]. An HFD recruits proinflammatory macrophages, which increase intestinal inflammation and permeability, leading to increased inflammatory cytokine and lipopolysaccharide (LPS) levels in the portal vein. These are circulated to the peripheral insulin-responsive tissues, leading to the enhancement of chronic inflammation in the liver and adipose tissue and the onset of insulin resistance. Guo et al. reported that Roux-en-Y gastric bypass could reduce the inflammation, which was associated with improvement of tight junction integrity and the intestinal barrier [12]. In this study, the focus was on the correlation between gut inflammation and insulin resistance.

In previous results, DJB surgery showed improvements in insulin resistance and nonalcoholic steatohepatitis (NASH) compared with the sham and liraglutide groups. In this result, on the one hand, the sham and liraglutide groups showed high levels of inflammatory cytokines in the intestine and low expressions of claudin-1 in the distal small intestine. On the other hand, the DJB group showed low inflammation and high claudin-1, which might correlate with an improvement in insulin resistance.

HFD-fed gut microbiota were reported to induce intestinal inflammation and high intestinal permeability [6], and the authors had also previously reported that DJB surgery changed the composition of gut microbiota, that is, it results in an increase in Gammaproteobacteria and a decrease in Bacteroidia [5]. These changes in gut microbiota resulting from DJB surgery might correlate with a suppression of gut inflammation.

A limitation of this study was the small number of rats used. Consequently, further investigation of the correlations between gut inflammation and permeability after metabolic surgery is needed.

In conclusion, DJB surgery might maintain gut permeability via suppression of gut inflammation. Therefore, DJB might improve insulin resistance by the suppression of inflammation in insulin-target tissues such as the liver and adipose tissue.

Compliance with Ethical Standards All applicable institutional and/or national guidelines for the care and use of animals were followed. Informed consent statement does not apply to this study.

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

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