Impact of Daikenchuto (TU-100) on The Early Postoperative Period in Duodenal-Jejunal Bypass

Shohei Okikawa
Department of Surgery, Tokushima University
Hideya Kashihara (kashihara.hideya@tokushima-u.ac.jp)
Department of Surgery, Tokushima University
Yuji Morine
Department of Surgery, Tokushima University
Mitsuo Shimada
Department of Surgery, Tokushima University
Kozo Yoshikawa
Department of Surgery, Tokushima University
Jun Higashijima
Department of Surgery, Tokushima University
Takuya Tokunaga
Department of Surgery, Tokushima University
Masaaki Nishi
Department of Surgery, Tokushima University
Chie Takasu
Department of Surgery, Tokushima University
Mitsue Nishiyama
Tsumura Kampo Research Laboratories, Tsumura & Co., Ami
Makoto Zushi
Tsumura Kampo Research Laboratories, Tsumura & Co., Ami

Research Article

Keywords: TU-100, Duodenal-jejunal bypass (DJB), inflammatory cytokines, Bifidobacterium, Blautia, Turicibacter

Posted Date: February 16th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-192416/v1

Abstract

Introduction: We investigated the effect of Daikenchuto (TU-100) on the early postoperative period in duodenal-jejunal bypass (DJB).

Methods: Study 1: The effect of TU-100 on diabetic rats was investigated. Rats were sacrificed after receiving TU-100 for one week. Study 2: The effect of TU-100 on DJB was investigated. Rats in the DJB and TU-100 treated DJB groups were sacrificed 24 hours postoperation to evaluate blood glucose, cytokine expression, and gut microbiome.

Results: Study 1: TU-100 did not affect glucose or body weight. TU-100 suppressed intestinal inflammation and modified the gut microbiome. Specifically, *Bifidobacterium* and *Blautia* were increased, and *Turicibacter* were decreased in this group. Study 2: Both DJB and TU-100 treated DJB rats showed lower blood glucose at 24 hours postoperation than at preoperation. Cytokine expression in the liver and small intestine of the TU-100 treated DJB group was significantly lower than that of the DJB group. The gut microbiome composition in TU-100 treated DJB rats was altered. In particular, *Bifidobacterium* and *Blautia* were increased in this group.

Conclusion: DJB suppressed blood glucose during the early postoperative period. TU-100 may enhance the anti-diabetic effect of metabolic surgery by changing the gut microbiome and suppressing inflammation in the early postoperative period.

Introduction

The incidence of type 2 diabetes mellitus (T2DM) and obesity is rapidly increasing worldwide [1]. Obesity is recognized as one of the major risk factors for T2DM, coronary artery disease, stroke, sleep apnea, and cancer [2–4]. It is well-known that severe obesity leads to health complications, decreases life expectancy, and influences patients' quality of life. Therefore, obesity and obesity-related diseases impose a burden on global health systems, societies, families, and affected individuals [5, 6].

Bariatric/metabolic surgeries (vertical sleeve gastrectomy, Roux-en-Y gastric bypass, laparoscopic adjustable gastric banding, and biliopancreatic diversion) achieved weight loss and improved obesity-related diseases [7]. Some considered bariatric interventions as metabolic surgery when the purpose was to improve metabolic disorder rather than weight loss alone, and these procedures could lead to changes of the gut microbiome and hormones, bile acid metabolism, and other factors that influenced glucose homeostasis independent of weight loss [8].

Duodenal-jejunal bypass (DJB) was a metabolic surgery involving exclusion of the duodenal and proximal jejunal from nutrients, jejunal Roux-en-Y reconstruction, and early nutrient delivery to the distal small intestine. Rubino et al. revealed substantial improvements in glucose homeostasis after DJB surgery [9].

In our previous report, DJB improved T2DM and liver steatosis by enhancing glucagon-like peptide-1 (GLP-1) secretion through increased bile acids and the proliferation of L cells in the ileum. Thus, we concluded that DJB surgery might be a key strategy for treating obese patients with T2DM [10]. Furthermore, we reported that DJB changed the composition of gut microbiota, and these changes might contribute to the beneficial effects of DJB [11]. In addition, DJB surgery was reported to maintain gut permeability through suppressing gut inflammation in our previous study [12]. DJB surgery may improve diabetes and liver steatosis by modifying the gut microbiota, maintaining gut permeability, and suppressing gut inflammation.

Seki et al. reported that a patient who underwent sleeve gastrectomy with DJB showed a glucose level below 200 mg/mL 100% of the time after postoperative day 3 (POD3) and did not receive diabetic medication on or after POD2 [13]. However, the mechanism underlying the improvement in diabetes during the early postoperative period remains unclear.

Daikenchuto (TU-100), a traditional Japanese medicine (a 'Kampo' medicine), was widely used Kampo medicine in Japan and had the greatest amount of preclinical and clinical evidence for improving postoperative gastrointestinal motility and preventing postoperative ileus [14–19]. We previously reported that TU-100 ameliorated colonic microvascular disorder in a rat model of Crohn's disease [20], prevented BT in fasting rats [21] and a CPT-11-induced intestinal injury model [22], and reduced the intestinal upregulation of cytokines, such as IL-6, TNF-α, and IL-1β. TU-100 maintained the diversity of the gut microbiota and decreased the abundance of *Erysipelotrichaceae* species, which are related to inflammatory diseases, such as colitis [23].

Therefore, we hypothesized that TU-100 might enhance the anti-diabetic effect of metabolic surgery by modifying the gut microbiome and suppressing inflammation during the early postoperative period. The purpose of this study was to investigate the effect of Daikenchuto (TU-100) on the early postoperative period in DJB using diabetic rats.

Materials And Methods

Animals

We used twelve 12-week-old male Goto–Kakizaki rats (CREA Japan, Inc.). These rats were reared in a controlled environment. The procedures in this experiment were approved by the Department of Animal Research Resources, Institute of Health Biosciences, Tokushima University. All experiments were performed according to the relevant guidelines and regulations, and all authors complied with the ARRIVE guidelines.

Methods

DJB, sham operation, TU-100 treatment, and TU-100 treated DJB

The 12 twelve rats were divided into four groups: a sham group, a DJB group, a Daikenchuto (TU-100) treated group, and a TU-100 treated DJB group. Each group had three rats. These rats were fasted before the surgery and anesthetized in 2% to 3% isoflurane and air/oxygen. In the sham operated group, a midline laparotomy was performed, and this incision was simply closed. DJB was performed as reported by Rubino, et al. [9]. Briefly, DJB was performed by post-pyloric transection of the duodenum, closure of the duodenal stump, transection of the jejunum at 10 cm from the Treitz ligament, reconstruction of the alimentary passage by duodenojejunostomy, and finally reconstruction of the biliopancreatic limb to the jejunum at 15 cm distal from the duodenojejunostomy. These rats in the sham and DJB group were allowed free access to water and a basal diet, freely (MF; Oriental Yeast, Tokyo, Japan). In the TU-100 group and TU-100 treated DJB group, TU-100 mixed food by gavage for 7 days before surgery.

Blood glucose in the DJB group was measured at preoperation and 24 hours postoperation. Blood glucose in the TU-100 group was measured at pre-TU-100 treatment and post-TU-100 treatment. In the TU-100 treated DJB group, blood glucose was measured at pre-TU-100 treatment, preoperation, and 24 hours postoperation.

Body weight was measured at pre-TU-100 treatment and post-TU-100 treatment in the TU-100 group and pre-TU-100 treatment, preoperation, and 24 hours postoperation in the TU-100 treated DJB group.

Fecal pellets were harvested at pre-TU-100 treatment and post-TU-100 treatment from the TU-100 group and pre-TU-100 treatment, preoperation, and 24 hours postoperation from the TU-100 treated DJB group.

Rats in the DJB and TU-100 treated DJB groups were sacrificed 24 hours postoperation.

Figures 1 and 5 show the experimental overview of Studies 1 and 2.

Reagents

TU-100 granules were made by Tsumura & Co (Tsumura Daikenchuto Extract Granules; Tsumura & Co, Tokyo, Japan). TU-100 contains three medical herbs: processed ginger (Zingiberis Siccatum Rhizoma), ginseng (Ginseng radix), and Japanese pepper (Zanthoxylum fruit).

Ribonucleic acid (RNA) isolation and quantitative real-time reverse transcription (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 RT-PCR was performed using a 7500 Real-Time PCR system with the TaqMan Gene Expression Assay-on-Demand and TaqMan Universal Master Mix (Applied Biosystems). The levels of the liver and gut inflammatory cytokines IFNγ, IL1, IL6, and TNFα (IFNγ: Rn00594078_m1; IL1: Rn00580432_m1; IL6: Rn00561420_m1; and TNFα: Rn00562055_m1) (Applied Biosystems) were assayed, and the control gene was the TaqMan Rat GAPDH endogenous control (GAPDH; 4352338E; Applied Biosystems). The thermocycling conditions were as follows: 2 minutes at 50°C, 10 minutes at

95°C, 40 cycles of fifteen 15 seconds at 95°C, and 1 minute at 65°C. Data were analyzed using Applied Biosystems Prism 7500 Sequence Detection System Software version 1.3.1.

16S rRNA gene metagenome sequencing of stool samples

Each 10–30 mg stool sample was treated with 1 mL extraction buffer [400 μL 10% sodium dodecyl sulfate (Sigma-Aldrich Japan Inc., Tokyo, Japan) in Tris-EDTA (TE) buffer (10 mmol/L Tris (pH 7.4; Fujifilm Waco Pure Chemical Corporation, Osaka, Japan) and 1 mmol/L EDTA (pH 8.0; Fujifilm Waco Pure Chemical Corporation))], 400 μL phenol:chloroform:isoamyl alcohol (25:24:1 v/v) (Nippon Gene, Tokyo, Japan), and 200 μL 3 M sodium acetate (Fujifilm Waco Pure Chemical Corporation), added to a Lysing Matrix E tube (MP Biomedicals, Solon OH, USA), and homogenized using a FastPrep-24 automated cell disruptor (MP Biomedicals) at a speed setting of 6 meters/sec for 40 sec. The homogenization process was repeated twice. The homogenate was centrifuged at 10,000 × g for 30 min, and DNA extract was obtained as the aqueous phase, which was purified twice by adding an equal volume of phenol:chloroform:isoamyl alcohol (25:24:1, v/v). Subsequently, an equal volume of isopropyl alcohol (Fujifilm Waco Pure Chemical Corporation) was added, and DNA was obtained as a pellet by centrifugation (10,000 × g, 5 min). After drying, the DNA was dissolved in TE. The preparation of the 16S rRNA gene metagenome library for MiSeq (Illumina, Inc., San Diego, USA) was performed according to the manufacturers' protocol. Briefly, 10 ng DNA template were amplified using an Advantage-HF 2 PCR kit (Takara Bio Inc., Shiga, Japan) with universal primers for the 16S rRNA v3–v4 region (forward primer: 5' TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG 3'; reverse primer: 5' GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC 3'). Subsequently, index sequences for each sample were added to both ends of the purified PCR fragments. The concentrations of each amplicon were measured using a Quant-iT PicoGreen dsDNA Assay Kit (Thermo Fisher Scientific, Inc., Waltham, USA) and mixed equally. The library was applied to a MiSeq Reagent Kit v3 (Illumina, Inc.), and sequences were determined in accordance with the manufacturers' standard protocol. Sequence data were processed using the 16S rRNA sequence analysis pipeline QIIME 1.8.0 as follows [23]. Initially, both sequence reads were joined, and sequences with a Phred quality score below 20 were removed. Chimera elimination by Usearch was performed to remove contaminated sequences. Open reference operational taxonomic unit (OTU) picking was performed against Greengenes 13_8 97% OTU representative sequences. A summary of the taxonomy of each sample was obtained using the script 'summarize_taxonomy_through_plots.py' in QIIME 1.8.0.

Statistical analysis

Data were analyzed using an unpaired Student's t-test or a one-way ANOVA with Bonferroni's post hoc test. Differences were defined as significant when $p < 0.05$. Results were expressed as means ± standard error of the mean (SEM). All data were generated using StatView version 5.0 for Windows (SAS Institute Inc., Cary, NC, USA).

Results

Effect of TU-100 on non-obese diabetic rats

Study 1: The effect of TU-100 on non-obese diabetic rats was investigated (Figure 1). During the first week, TU-100 had no effect on blood glucose or body weight (Figure 2a,b). However, TU-100 suppressed the levels of IL-1β and IL-6 in the proximal small intestine, TNFα in the middle small intestine, and TNFα and IFN-γ in the distal small intestine (Figure 3a–e). Therefore, this indicated that TU-100 suppressed gut inflammation. In the PCA analysis, significant alterations in the composition of the gut microbiome in TU-100 treated rats were observed (Figure 4a,b). In particular, *Bifidobacterium* and *Blautia* were increased, and the *Turicibacter* genus was decreased in the TU-100 group (Figure 4c–e).

Effect of TU-100 on duodenal-jejunal bypassed non-obese diabetic rats

Study 2: The effect of TU-100 on duodenal-jejunal bypassed non-obese diabetic rats was investigated (Figure 5). Both DJB and TU-100 treated DJB rats showed lower blood glucose at 24 hours postoperation than that measured preoperation (Figure 6a,b). However, both the DJB and TU-100 treated DJB groups exhibited body weight changes after 24 hours (Figure 6c,d). Cytokine expression in the liver and small intestine of the TU-100 treated DJB group was significantly lower than that of the DJB group (Figure 7a–d), indicating that preoperative treatment with TU-100 may suppress postoperative inflammation after DJB. In addition, PCA analysis revealed significant changes in the composition of the gut microbiome in TU-100 treated DJB rats. However, there was no difference in the gut microbiome between preoperation and postoperation samples in the DJB group (Figure 8a–c). In particular, *Bifidobacterium* and *Blautia* were increased in the TU-100 treated DJB group. In the TU-100 treated DJB group, the proportion of *Turicibacter* after DJB was significantly decreased compared with that before DJB (Figure 8d–f).

Discussion

This study investigated the effect of Daikenchuto (TU-100), a traditional Japanese (Kampo) medicine, on the early postoperative period in DJB using non-obese diabetic rats. Blood glucose levels at 24 hours postoperation in the TU-100 treated DJB group were significantly lower than those before TU-100 treatment and surgery. Cytokine expression in the liver and small intestine of the TU-100 treated DJB group was significantly lower than in the DJB group. The gut microbiome composition in TU-100 and TU-100 treated DJB rats was significantly altered. Specifically, *Bifidobacterium* and *Blautia* were increased, and the proportion of *Turicibacter* was decreased in the TU-100 and TU-100 treated DJB groups. Therefore, TU-100 may enhance the anti-diabetic effect of metabolic surgery by modifying the gut microbiome and suppressing inflammation during the early postoperative period. To our knowledge, this is the first report to reveal the anti-diabetic mechanism of DJB in the early postoperative period and the usefulness of TU-100 during perioperative care for metabolic surgery.

Metabolic surgery is recommended for the treatment of T2DM patients because it leads to normalization of hyperglycemia within days after surgery, an effect that is thought to be independent of weight loss [24, 25]. Metabolic surgery positively modifies the gut endocrine system to decrease appetite and improve glucose metabolism [26]. After RYGB, ingested food empties rapidly from the small gastric pouch into the

alimentary limb and mixes with the biliary and pancreatic exocrine secretions in the common limb. The rapid emptying of the reduced gastric pouch results in accelerated nutrient transit [27, 28] and alters the post-prandial gastrointestinal hormonal chain of events. This enhances the release of satiety hormones, such as cholecystokinin (CCK) [29, 30], peptide yy (PYY) [31], GLP-1 [32–34], and oxyntomodulin [35]. We reported that DJB surgery improved diabetes and non-alcoholic steatohepatitis by enhancing GLP-1 secretion through increased bile acids and the proliferation of L cells in the ileum [10].

Seki et al. reported that a patient who underwent sleeve gastrectomy with DJB showed an early blood glucose suppressive effect after POD3 [13]. In our previous report, we also investigated the effect of Roux-en-Y reconstruction on T2DM with gastric cancer in the early postoperative period. Roux-en-Y reconstruction achieved early improvement in T2DM regardless of any body weight loss [36]. However, the mechanism underlying the improvement in diabetes during the early postoperative period remains unclear.

It is well-known that a high-fat diet (HFD) induces chronic intestinal inflammation and insulin resistance. Kawano Y et al. reported the association between intestinal inflammation and insulin resistance [37]. Exposure to a HFD results in the recruitment of pro-inflammatory macrophages, which promote intestinal inflammation and permeability, leading to increased inflammatory cytokine and lipopolysaccharide levels in the portal vein. Subsequently, these are circulated to the peripheral insulin-responsive tissues, leading to increased chronic inflammation in the liver and adipose tissue and the onset of insulin resistance. In our previous report, DJB surgery was shown to maintain gut permeability via the suppression of gut inflammation [12].

Gut microbiota influence host health, and an imbalanced bacterial population associated with a HFD has been shown to trigger the development of metabolic diseases, such as obesity [38] and diabetes [39], in animal models. We reported that DJB surgery changed the composition of the gut microbiota, and these changes might contribute to the positive effects of DJB [11]. However, the effect of DJB on the gut microbiome and inflammation in the early postoperative period remains unclear.

Therefore, we focused on TU-100 as a potential enhancer of the effect of metabolic surgery during the early postoperative period. TU-100, a traditional Japanese medicine (a ‘Kampo’ medicine), is the most widely used Kampo medicine in Japan and has the greatest amount of preclinical and clinical evidence for improving gastrointestinal motility and preventing postoperative ileus after abdominal surgery [14-19]. In our previous reports, TU-100 prevented BT in fasting rats [21] and a CPT-11-induced intestinal injury model [22] and reduced the upregulation of cytokines in the intestine. Furthermore, TU-100 maintained the diversity of the gut microbiota and decreased the abundance of *Erysipelotrichaceae* species, which are associated with inflammatory conditions, such as colitis [23].

In this study, the TU-100 treated DJB group showed stable blood glucose levels and suppression of inflammatory cytokines in the liver and intestine. Therefore, TU-100 may enhance the anti-diabetic effect of DJB by suppressing inflammation in the early postoperative period. In addition, this suppression of

inflammation in the liver and intestine was attributed to changes in the gut microbiome following pre-treatment with TU-100.

In this study, *Bifidobacterium* and *Blautia* were increased, and the proportion of *Turicibacter* was decreased in the TU-100 and TU-100 treated DJB groups. *Bifidobacterium* species are reportedly associated with improvements in glucose intolerance, insulin secretion, and the reduction of inflammatory cytokines in plasma and fat tissues [39]. *Blautia* species are known to produce the short-chain fatty acid (SCFA) butyrate. SCFAs suppress inflammation by regulating the immune system and inflammatory cytokines [40]. The genus *Blautia* is less prominent in diabetic adults, pediatric patients [41, 42], and those with other diseases, such as liver cirrhosis, rectal cancer, and rheumatoid arthritis [43, 44]. In contrast, *Turicibacter* species are associated with reduced insulin sensitivity, increased inflammation in white adipose tissue, and the induction of obesity [45]. These changes in the gut microbiome may induce early postoperative improvement in diabetes via the suppression of gut inflammation. Therefore, we recommend preoperative TU-100 treatment to facilitate postoperative care in metabolic surgery.

The present study has several limitations. First, we used non-obese diabetic rats in this study. These rats were used to ensure that there was no influence of body weight. In a future study, diet-induced obese rats will be used. Second, the direct relationships among TU-100, the gut microbiome, and inflammation remain unclear. To address this, the supplementation of *Bifidobacterium* and *Blautia* will be performed in a future study.

In conclusion, DJB suppressed blood glucose in the early postoperative period. TU-100 may enhance the anti-diabetic effect of metabolic surgery by modifying the gut microbiome and suppressing inflammation during the early postoperative period.

Declarations

Acknowledgments

The authors are thankful to the staff of the Department of Surgery, Institute of Health Biosciences, Tokushima University and Tsumura Research Laboratories, Tsumura & Co., Ami, Ibaraki, Japan. We thank Melissa Crawford, PhD, from Edanz Group (https://en-author-services.edanz.com/ac) for editing a draft of this manuscript.

Author contributions

Hideya Kashihara, Yuji Morine, and Mitsuo Shimada contributed to the conception or design of the work, Shohei Okikawa, Hideya Kashihara Mitsue Nishiyama, and Makoto Zushi contributed to the acquisition, analysis, or interpretation of data, Kozo Yoshikawa contributed to the creation of new software used in the work, and Hideya Kashihara and Yuji Morine drafted the work or substantively revised it. All authors approved the submitted version (and any substantially modified version that involves the author's contribution to the study) and agreed both to be personally accountable for their own contributions and

ensure that questions related to the accuracy or integrity of any part of the work, even ones in which the author was not personally involved, are appropriately investigated, resolved, and the resolution documented in the literature.

Additional information

Competing interest statement

Mitsuo Shimada received a research grant from Tsumura & Co.

Mitsue Nishiyama and Makoto Zushi are employed by Tsumura & Co.

Other authors declare no potential conflict of interest for this article.

Data availability statement

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

References

1. Islam, S.M. *et al*. Non-communicable diseases (NCDs) in developing countries: a symposium report. *Global Health*. **10**, 81; 10.1186/s12992-014-0081-9 (2014).
2. Lavie, C.J., Milani, R.V. & Ventura, H.O. Obesity and cardiovascular disease: risk factor, paradox, and impact of weight loss. *Am. Coll. Cardiol.* **53**, 1925–1932 (2009).
3. Koh-Banerjee, P. *et al*. Changes in body weight and body fat distribution as risk factors for clinical diabetes in US men. *J. Epidemiol*. **159**, 1150–1159 (2004).
4. Dobbins, M., Decorby, K. & Choi, B.C. The association between obesity and cancer risk: a meta-analysis of observational studies from 1985 to 2011. *ISRN Prev. Med*. **2013**, 680536 (2013).
5. Chan, R.S. & Woo J. Prevention of overweight and obesity: how effective is the current public health approach. *J. Environ. Res. Public Health*. **7**, 765–783 (2010).
6. Shariful Islam, S.M. *et al*. Healthcare use and expenditure for diabetes in Bangladesh. *BMJ Glob. Health*. **2**, e000033 (2017).
7. Chang, S.H. *et al*. The effectiveness and risks of bariatric surgery. *JAMA Surg*. **149**, 275–287 (2014).
8. Francesco, R. *et al.* Metabolic surgery in the treatment algorithm for type 2 diabetes. *Diabetes Care*. **39**, 861–877 (2016).
9. Rubino, F. *et al*. The mechanism of diabetes control after gastrointestinal bypass surgery reveals a role of the proximal small intestine in the pathophysiology of type 2 diabetes. *Surg.* **244**, 741–749 (2006).

10. Kashihara, H. *et al.* Duodenal-jejunal bypass improves diabetes and liver steatosis via enhanced glucagon-like peptide-1 elicited by bile acids. *Gastroenterol. Hepatol.* **30**, 308–15 (2015).
11. Kashihara, H. *et al*. Duodenal-jejunal bypass changes the composition of the gut microbiota. *Today*. **47**, 137–140 (2017).
12. Kashihara, H. *et al.* Duodenal-jejunal bypass maintains gut permeability by suppressing gut inflammation. *Surg*. **29**, 2745–2749 (2019).
13. Seki, Y., Kasama, K., Umezawa, A. & Kurokawa, Y. Laparoscopic sleeve gastrectomy with duodenojejunal bypass for type 2 diabetes mellitus. *Obes. Surg.* **26**, 2035–2044 (2016).
14. Kono, T., Kanematsu, T. & Kitajima, M. Exodus of Kampo, traditional Japanese medicine, from the complementary and alternative medicines: is it time yet?**146**, 837–840 (2019).
15. Manabe, N. *et al.* Effect of daikenchuto (TU-100) on gastrointestinal and colonic transit in humans. *J. Physiol. Gastrointest. Liver Physiol.* **298**, G970–G975 (2010).
16. Iturrino, J. *et al*. Randomised clinical trial: the effects of daikenchuto, TU-100, on gastrointestinal and colonic transit, anorectal and bowel function in female patients with functional constipation. *Pharmacol. Ther.* **37**, 776–785 (2013).
17. Okada, K. *et al.* Effect of Daikenchuto (TJ-100) on postoperative bowel motility and on prevention of paralytic ileus after pancreaticoduodenectomy: a multicenter, randomized, placebo-controlled phase II trial (the JAPAN-PD study). *J. Clin. Oncol.* **43**, 436–438 (2013).
18. Shimada, M. *et al.* Effect of TU-100, a traditional Japanese medicine, administered after hepatic resection in patients with liver cancer: a multi-center, phase III trial (JFMC40- 1001). *J. Clin. Oncol.* **20**, 95–104 (2015).
19. Yoshikawa, K. *et al.* Effect of Daikenchuto, a traditional Japanese herbal medicine, after total gastrectomy for gastric cancer: a multicenter, randomized, double-blind, placebo controlled, phase II trial. *Am. Coll. Surg.* **221**, 571–578 (2015).
20. Kono, T. *et al.* Daikenchuto (TU-100) ameliorates colon microvascular dysfunction via endogenous adrenomedullin in Crohn's disease rat model. *Gastroenterol*. **46**, 1187–1196 (2011).
21. Yoshikawa, K. *et al.* Kampo medicine "Daikenchu-to" prevents bacterial translocation in rats. *Dis. Sci.* **53**, 1824–1831 (2008).
22. Chikakiyo, M. *et al.* Kampo medicine "Dai-kenchu-to" prevents CPT-11-induced small-intestinal injury in rats. *Today*. **42**, 60–67 (2012).
23. Yoshikawa, K. *et al.* Effect of Kampo medicine"Dai-kenchu-to" on microbiome in the intestine of the rats with fast stress. *Med. Invest.* **60**, 221–227 (2013).
24. Schauer, P.R. *et al*. Effect of laparoscopic Roux-en Y gastric bypass on type 2 diabetes mellitus. *Annals of Surgery.* **238**, 467–484 (2003).
25. Fried, M. *et al*. Metabolic surgery for the treatment of type 2 diabetes in patients with BMI <35 kg/m2: an integrative review of early studies. *Surgery.* **20**, 776–790 (2010).

26. Quercia, I., Dutia, R., Kotler, D.P., Belsley, S. & Laferrère B. Gastrointestinal changes after bariatric surgery. *Diabetes Metab.* **40**, 87–94 (2014).
27. Wang, G. *et al.* Accelerated gastric emptying but no carbohydrate malabsorption 1 year after gastric bypass surgery (GBP). *Surg.* **22**, 1263–1267 (2012).
28. Stano, S. *et al.* Effect of meal size and texture on gastric pouch emptying and glucagon-like peptide 1 after gastric bypass surgery. *Obes. Relat. Dis.* **13**, 1975–1983 (2017).
29. Steinert, R.E. *et al*. Ghrelin, CCK, GLP-1, and PYY(3–36): secretory controls and physiological roles in eating and glycemia in health, obesity, and after RYGB. *Rev.* **97**, 411–463 (2016).
30. Ockander, L., Hedenbro, J.L., Rehfeld, J.F. & Sjölund, K. Jejunoileal bypass changes the duodenal cholecystokinin and somatostatin cell density. *Surg.* **13**. 584–590 (2003).
31. Olivan, B. *et al.* Effect of weight loss by diet or gastric bypass surgery on peptide YY3-36 levels. *Surg.* **249**, 948–953 (2009).
32. Vidal, J. *et al.* Long-term effects of Roux-en-Y gastric bypass surgery on plasma glucagon-like peptide-1 and islet function in morbidly obese subjects. *J. Clin. Endocrinol. Metab.* **94**, 884–891 (2009).
33. le Roux, C.W. *et al.* Gut hormones as mediators of appetite and weight loss after Roux-en-Y gastric bypass. *Ann. Surg.* **246**, 780–785 (2007).
34. Morinigo, R. *et al.* Glucagon-like peptide-1, peptide YY, hunger, and satiety after gastric bypass surgery in morbidly obese subjects. *J. Clin. Endocrinol. Metab.* **91**, 1735–40 (2006).
35. Laferrère, B. *et al.* Rise of oxyntomodulin in response to oral glucose after gastric bypass surgery in patients with type 2 diabetes. *J. Clin. Endocrinol. Metab.* **95**, 4072–4076 (2010).
36. Kashihara, H. *et al*. The effect of Roux-en-Y reconstruction on type 2 diabetes in the early postoperative period. *Anticancer Res.* **38**, 4901–4905 (2018).
37. Kawano, Y. *et al.* Colonic pro-inflammatory macrophages cause insulin resistance in an intestinal Ccl2/Ccr2-dependent manner. *Cell Metab.* **24**, 295–310 (2016).
38. Hildebrandt, M.A. *et al.* High-fat diet determines the composition of the murine gut microbiome independently of obesity. *Gastroenterology.* **137**, 1716–1724 (2009).
39. Cani, P.D. *et al.* Selective increases of bifidobacteria in gut microflora improve high-fat-diet-induced diabetes in mice through a mechanism associated with endotoxaemia. *Diabetologia.* **50**, 2374–2783 (2007).
40. Li, L., Ma, L. & Fu P. Gut microbiota-derived short-chain fatty acids and kidney diseases. *Drug Des. Devel. Ther.* **11**, 3531–3542 (2017).
41. Larsen, N. *et al.* Gut microbiota in human adults with type 2 diabetes differs from non-diabetic adults. *PLoS ONE 5.* **5**, e9085 (2010).
42. Murri, M. *et al.* Gut microbiota in children with type 1 diabetes differs from that in healthy children: a case-control study. *BMC Med.* **11**, 46 (2013).

43. Kakiyama, G. *et al.* Modulation of the fecal bile acid profile by gut microbiota in cirrhosis. *J. Hepatol.* **58**, 949–955 (2013).
44. Ohigashi, S. *et al.* Changes of the intestinal microbiota, short chain fatty acids, and fecal pH in patients with colorectal cancer. *Dig. Dis. Sci.* **58**, 1717–1726 (2013).
45. Caesar, R., Tremaroli, V., Kovatcheva-Datchary, P., Cani, P.D. & Bäckhed, F. Crosstalk between gut microbiota and dietary lipids aggravates WAT inflammation through TLR signaling. *Cell Metab.* **22**, 658–668 (2015).

Figures

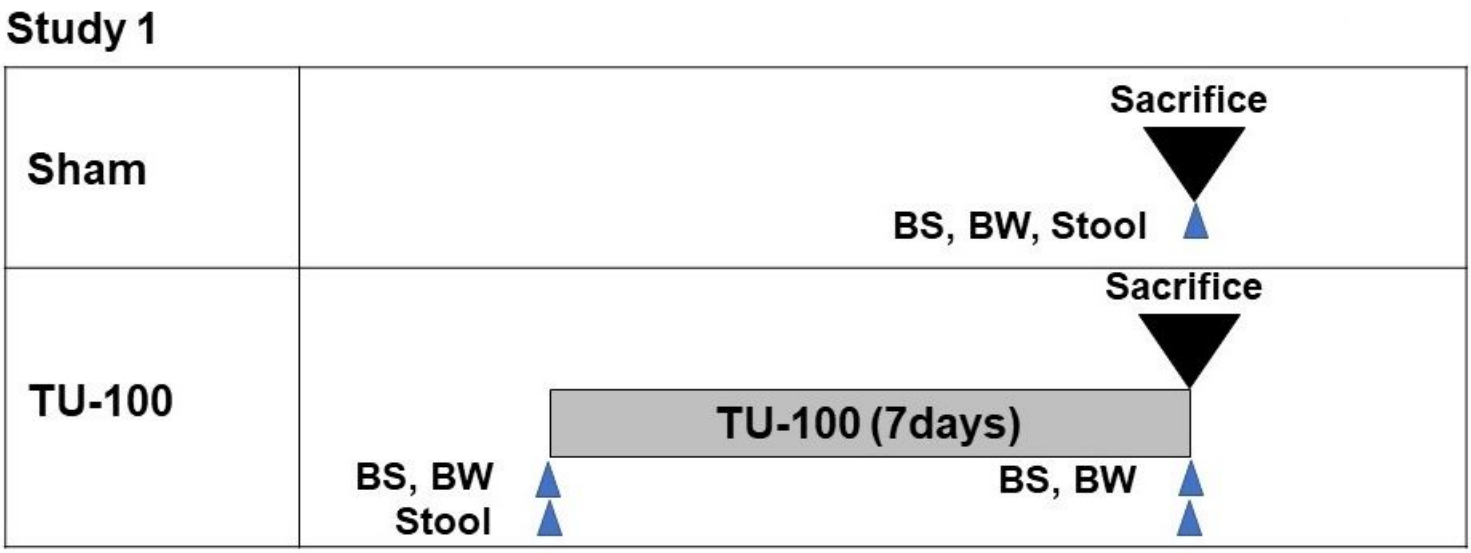

Figure 1

Experimental design for the sham and TU-100 groups.

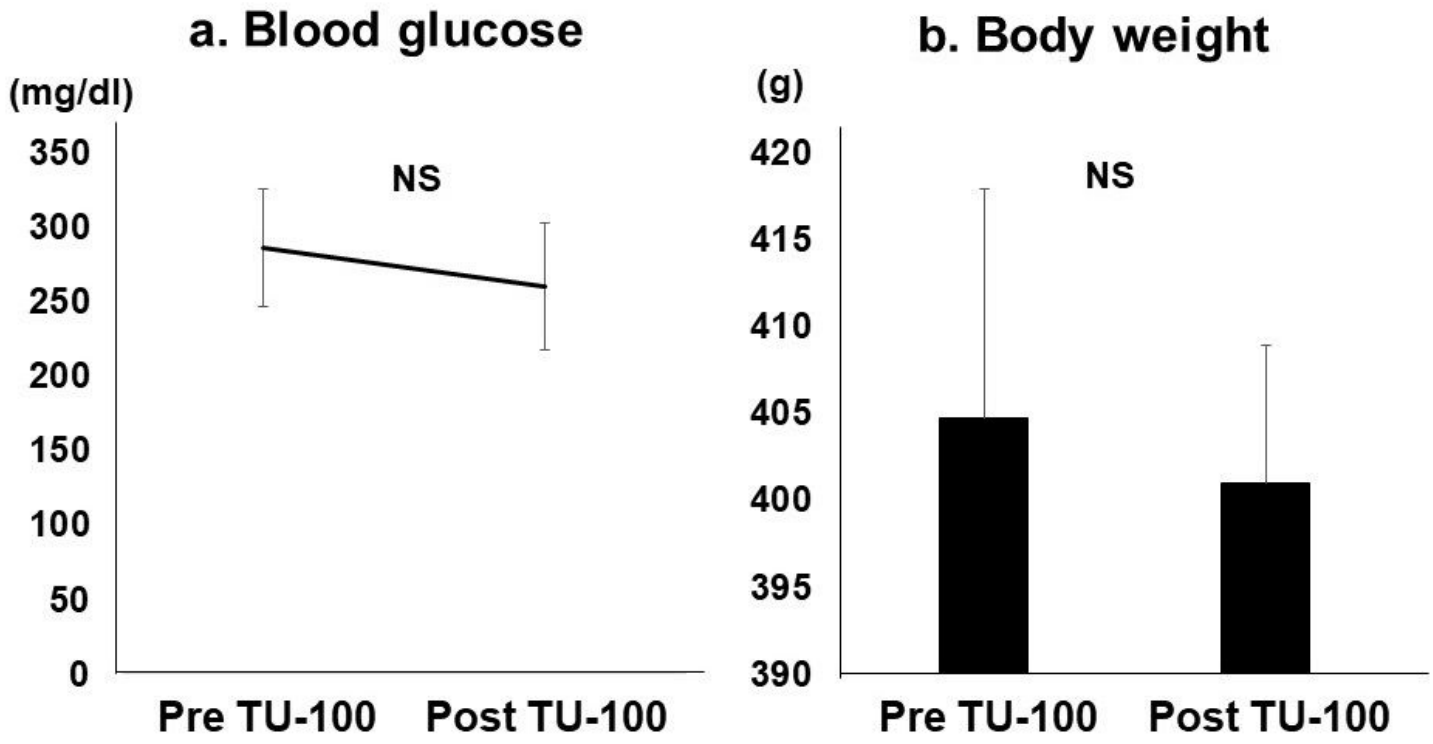

Figure 2

(a) Blood glucose changes at pre-TU-100 and post-TU-100 treatment. (b) Body weight changes at pre-TU-100 and post-TU-100 treatment.

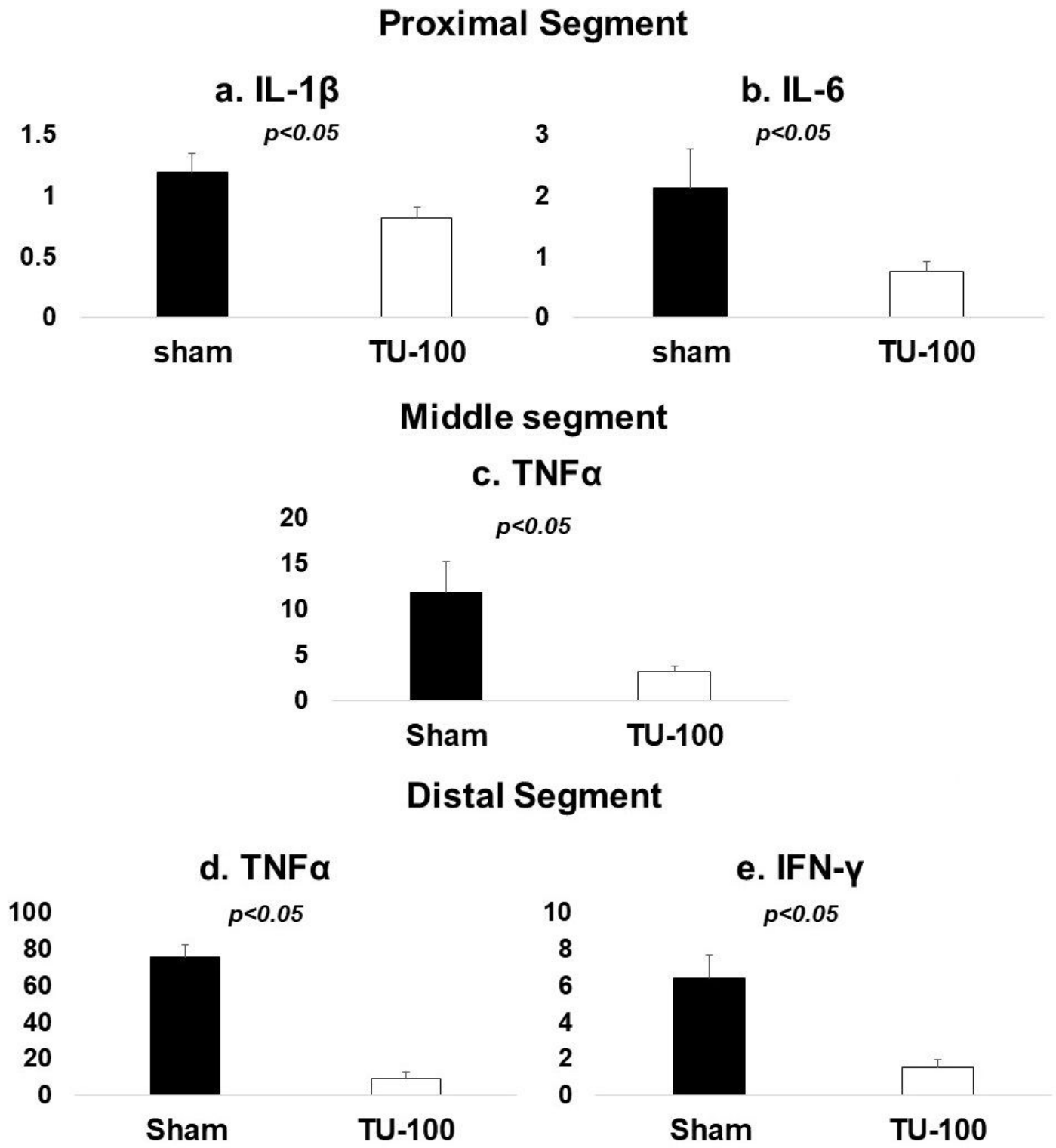

Figure 3

Expression of inflammatory cytokines in the small intestine of the sham and TU-100 group. Expression of IL-1β (a) and IL-6 (b) in the proximal small intestine, TNFα (c) in the middle small intestine, and TNFα (d) and IFN-γ (e) in the distal small intestine.

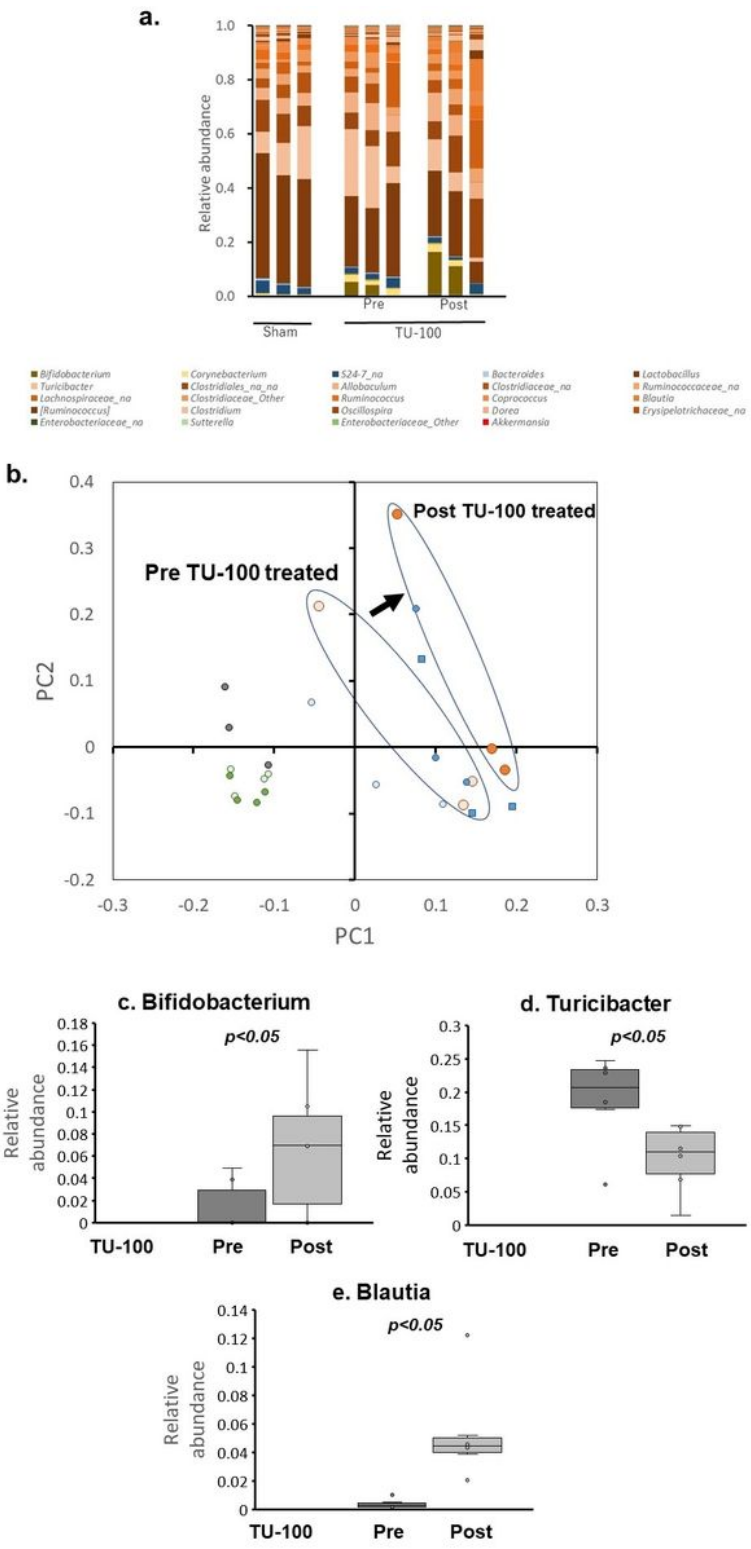

Figure 4

(a) Meta 16S rRNA gene sequencing analysis of the fecal microbiota in the sham and pre- and post-TU-100 treatment groups. (b) PCA analysis in the TU-100 group. Relative abundance of Bifidobacterium (c), Turicibacter (d), and Blautia (e) in the sham and TU-100 groups.

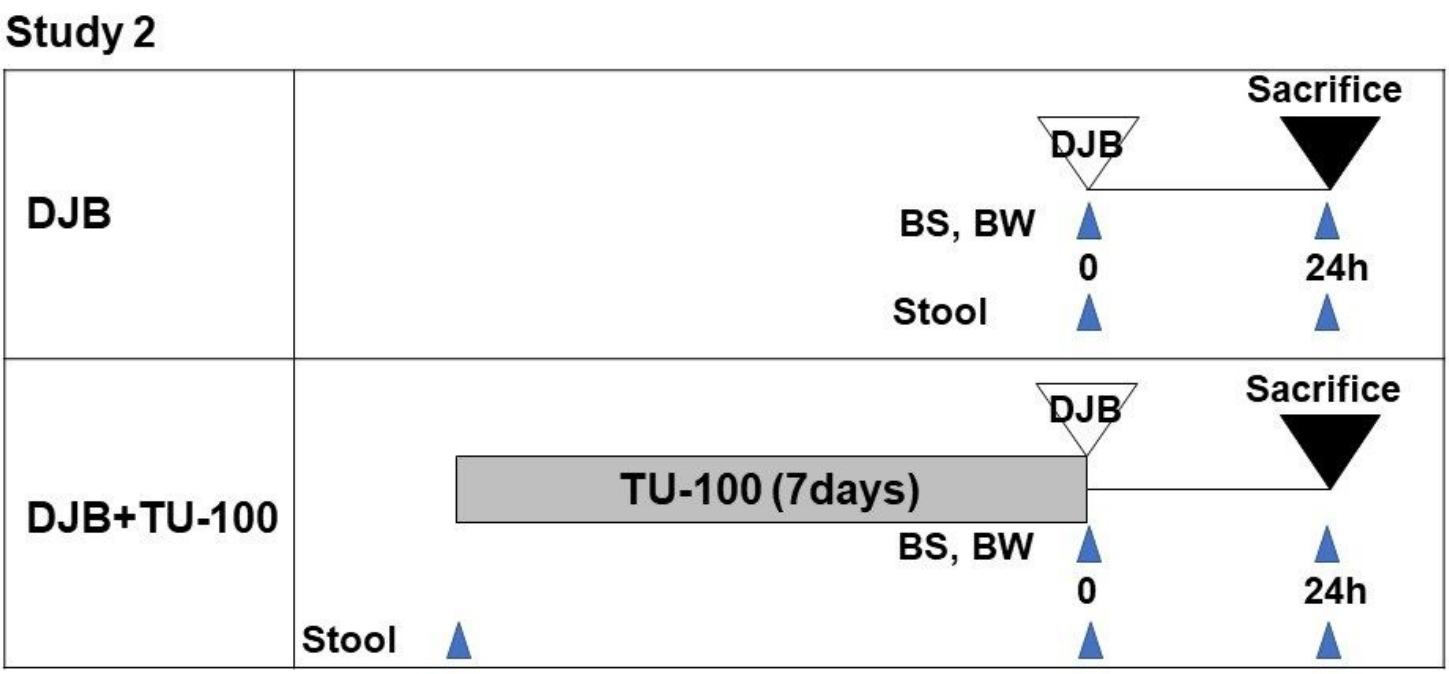

Figure 5

Experimental design in the DJB and TU-100 treated DJB groups.

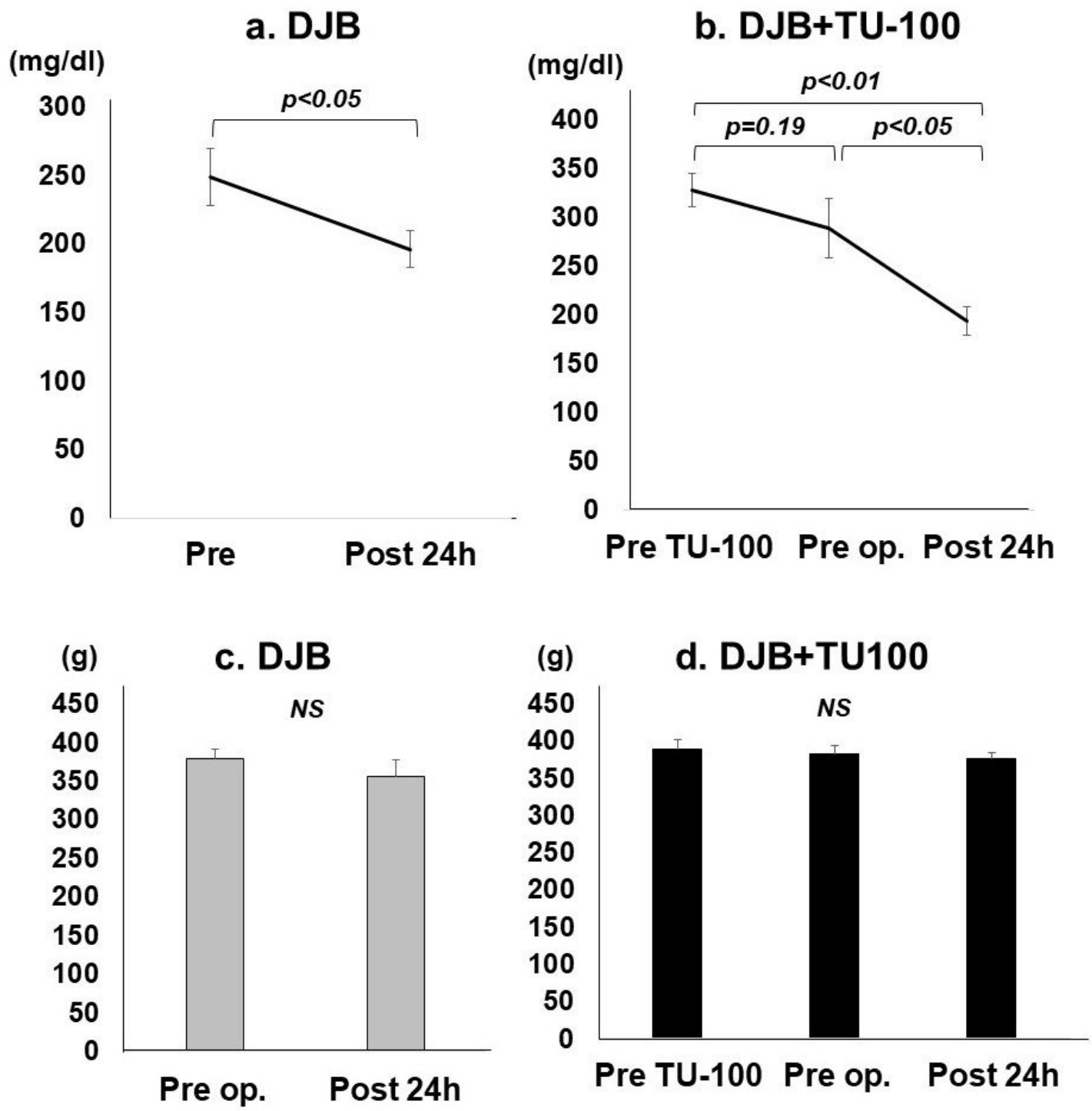

Figure 6

(a) Blood glucose changes at preoperation and 24 hours postoperation in the DJB group. (b) Blood glucose changes at pre-TU-100, preoperation, and 24 hours postoperation in the TU-100 treated DJB group. (c) Body weight changes at preoperation and 24 hours postoperation in DJB treated rats. (d) Body weight changes at pre-TU-100, preoperation, and 24 hours postoperation in the TU-100 treated DJB group.

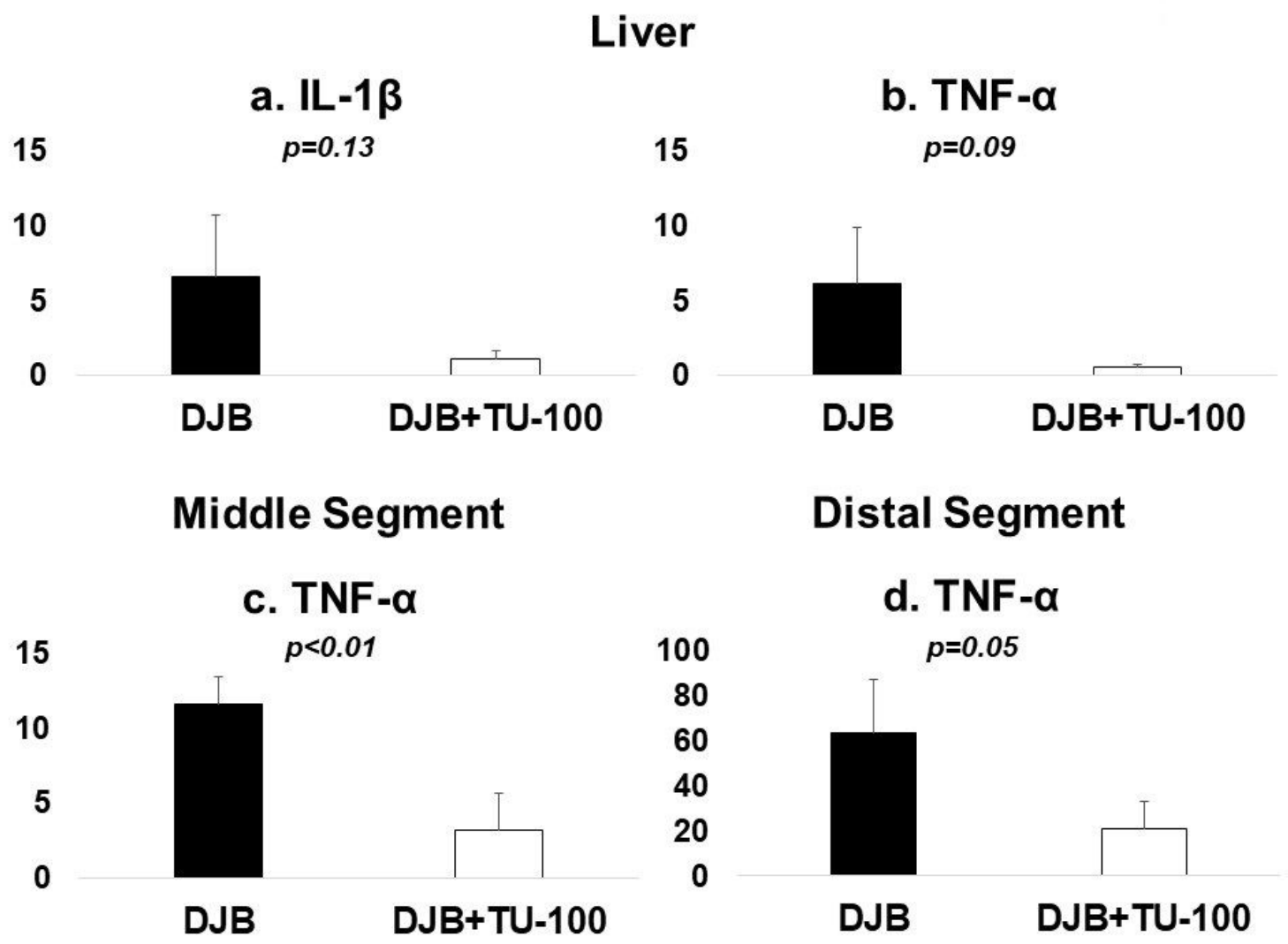

Figure 7

Expression of inflammatory cytokines in the liver and small intestine of the DJB and TU-100 treated DJB groups. Expression of IL-1β (a) and TNFα (b) in the liver, TNFα (c) in the middle small intestine, and TNFα (d) in the distal small intestine.

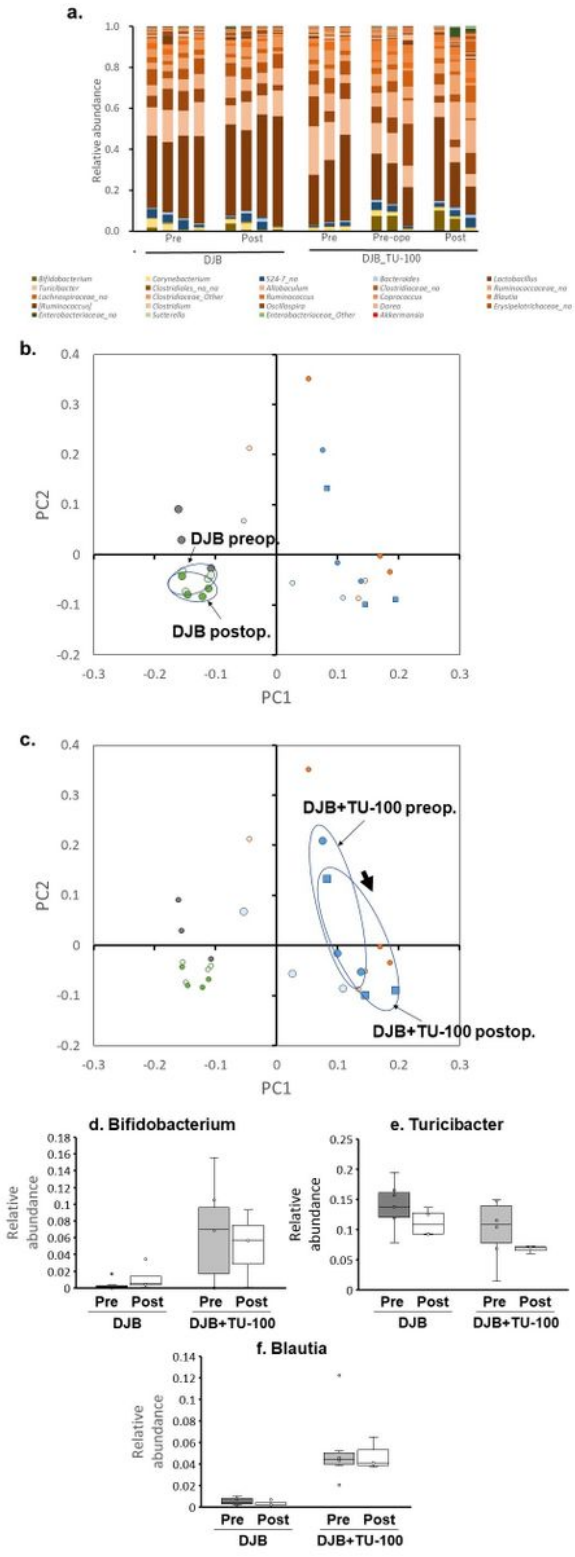

Figure 8

(a) Meta 16S rRNA gene sequencing analysis of the fecal microbiota in the DJB and TU-100 treated DJB groups. PCA analysis in the DJB (b) and TU-100 treated DJB groups (c). Relative abundance of Bifidobacterium (d), Turicibacter (e), and Blautia (f) in the DJB and TU-100 treated DJB groups.