The Effect and Mechanism of Duodenal-Jejunal Bypass to Treat Type 2 Diabetes Mellitus in a Rat Model

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**Keywords**
Duodenal-jejunal bypass · Glucagon-like peptide-1 · Endoplasmic reticulum stress · Apoptosis

**Abstract**

**Objectives:** Bariatric surgery can treat obesity and T2DM, but the specific mechanism is unknown. This study investigated the effect and possible mechanism of duodenal-jejunal bypass (DJB) to treat T2DM. **Methods:** A T2DM rat model was established using a high-fat, high-sugar diet and a low dose of streptozotocin. DJB surgery and a sham operation (SO) were performed to analyze the effects on glucose homeostasis, lipid metabolism, and inflammation changes. Furthermore, the glucagon-like peptide-1 (GLP-1) in the ileum and the markers of endoplasmic reticulum stress (ERS) in the pancreas were examined after the surgery. The insulinoma cells (INS-1) were divided into three groups; group A was cultured with a normal sugar content (11.1 mmol/L), group B was cultured with fluctuating high glucose (11.1 mmol/L alternating with 33.3 mmol/L), and group C was cultured with fluctuating high glucose and exendin-4 (100 nmol/L). The cells were continuously cultured for 7 days in complete culture medium. The viability of the INS-1 cells was then investigated using the MTT method, apoptosis was detected by flow cytometry, and the ERS markers were detected by Western blot. **Results:** The blood glucose, lipids, insulin, and TNF-α were significantly elevated in the T2DM model. A gradual recovery was observed in the DJB group. GLP-1 expression in the distal ileum of the DJB group was significantly higher than that in the T2DM control group (DM) and the SO group ($p < 0.05$), and the markers of ERS expression in the pancreases of the DJB group decreased significantly more than those of groups DM and SO ($p < 0.05$). Compared with group A, the cell viability in group B was decreased, and the ERS and apoptosis were increased ($p < 0.05$). However, compared with group B, the cell viability in group C was improved, and the ERS and apoptosis declined ($p < 0.05$). **Conclusions:** DJB can be used to treat T2DM in T2DM rats. The mechanism may be that the DJB stimulates the increased expression of GLP-1 on the far side of the ileum, and then, GLP-1 inhibits ERS in the pancreas, reducing the apoptosis of β cells to create a treatment effect in the T2DM rats.

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Effect and Mechanism of DJB to Treat T2DM

Introduction

The endoplasmic reticulum is the central cell organelle in eukaryotic cells during protein synthesis and folding, quality control, and transport location, and it can adjust its own capacity to adapt the intracellular synthesis, metabolism, and other physiological needs. In response to a variety of physiological and pathological stimuli, the endoplasmic reticulum folding capacity cannot meet the demand for the new synthesis of intracellular unfolded protein, causing unfolded and misfolded protein accumulation and an imbalance in Ca^{2+}, which is known as endoplasmic reticulum stress (ERS). Appropriate ERS is one of the self defense mechanisms available when the body faces a variety of physiological and pathological stimuli, but too strong or too long an ERS occurrence can lead to apoptosis, which then causes various diseases [1]. Ozcan suggested that the ERS is an important way to lead to apoptosis, which then causes various diseases [1]. Ozcan suggested that the ERS is an important way to cause diabetes, obesity, and insulin resistance (IR) [2]. ERS can activate the c-jun NH_{2}-terminal kinase pathway, leading to obesity, IR, and diabetes mellitus [3].

Gastric bypass (GBP) can be used to treat T2DM with morbid obesity [4, 5], and the modified version, the duodenal-jejunal bypass (DJB), can also be used to treat non-obese spontaneous diabetes in rats [6]. However, there is still a great deal of controversy about the effect of DJB on the treatment of nonobese T2DM patients [7–10]. The complete mechanisms through which GBP and DJB treat T2DM are not known.

At present, most scholars recognize the enteroinsular axis theory. There are two hypotheses about the regulation mechanism of the enteroinsular axis: (1) foregut hypothesis: food avoids the stimulation of gastroduodenum and reduces the release of IR factors and other substances [11, 12] and (2) hindgut hypothesis: food stimulation induces the synthesis and/or secretion of endogenous endocrine hormones in the intestine, regulates the endocrine function of the pancreas through the entero-islet axis, increases the synthesis and secretion of insulin (INS), and improves the sensitivity of peripheral tissues to INS [13, 14].

Recently, Wu et al. [15] found that decreased apoptosis and improved secretion function of the β cells were observed in DJB surgery group rats, and the NOD-like receptor family pyrin domain containing 3 (NLRP3) inflammasome pathway in infiltrating macrophages was also suppressed after DJB surgery. Moreover, diabetic remission acquired by DJB sustained in the NLRP3-knockdown macrophage reconstitution group, while extinguished in the group reconstituted with the wild-type macrophage. They draw a conclusion that deactivation of the NLRP3 inflammasome in infiltrating macrophages by DJB surgery mediates improvement of β cell function in T2DM [15].

Wang et al. [16] found animals undergoing DJB did not experience symptoms typical of uncompensated diabetes, including hyperphagia and progressive weight loss. After streptozotocin (STZ) injection and challenged by glucose load, glucose control and incretin response in the DJB group were better than those in the control and sham groups. They draw a conclusion that DJB is able to protect pancreatic beta cells from apoptosis, which leads to better glycemic control and A delayed onset of diabetes in STZ-induced diabetic rats [16].

Chai et al. [17] found that the excluded part of the ileum did not decrease the levels of glucagon-like peptide-1 (GLP-1) and peptide tyrosine-tyrosine amide after DJB, regarding that exclusion of the distal ileum cannot reverse the antidiabetic effects of DJB surgery. On the contrary, Patriti et al. [13] found modified secretion profile of GLP-1 in ileal transposition rats could be the result of the early arrival of glucose in the transposed ileum that triggers a longer secretion. Thus, we explore whether DJB can be used to treat T2DM by promoting the secretion of GLP-1 from the distal small intestine, inhibiting the ERS in the pancreas and protecting the β cells.

Materials and Methods

Animals and Cells

The details regarding the T2DM, sham operation (SO), and DJB animal models are explained in our previous article [18]. Four-week-old male Sprague-Dawley (SD) rats (weight, 80–100 g) were acquired from the Experimental Animal Center of Chongqing Medical University (Chongqing, China; animal license No. SYXK [Chongqing] 2012-0001). All the rats were acclimated to their SPF environment for 1 week prior to the beginning of the experiment. The rats were housed in standard polypropylene cages and maintained at a controlled room temperature (22 ± 2°C) and humidity (55 ± 5%) with a 12/12 h light/dark cycle. The T2DM model group rats received a high-fat, high-sugar diet (standard rat feed 60%, solid lard 10%, egg yolk powder 10%, granulated sugar 20%) for 4 weeks and were subsequently injected intraperitoneally with a low dose of STZ (30 mg/kg) dissolved in citrate solution (0.1 M citric acid and 0.2 M sodium phosphate, pH 4.2–4.5). The rats that were injected with STZ developed diabetes, as indicated by the levels of fasting blood glucose (FBG) of ≥7.8 mmol/L for 2 weeks following the injection [19]. Carefully observe the activity, hair, drinking water, eating, urination, and bedding of the rats. Before surgery, the rats with an FBG ≥7.8 mmol/L and blood glucose of ≥11.1 mmol/L after 2 h of the OGTT experiment were included in the experiment.

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The rats with T2DM were randomly divided into three groups: the DM group \( n = 6 \) that received no operation; the SO group \( n = 6 \) that received a SO, in which the transection and reanastomosis of the gastrointestinal tract were performed at the given sites (corresponding to where enterotomies were performed for the DJB), and the physiologic circuit of food was maintained through the bowel. When needed, the operation was prolonged to produce a similar degree of anesthesiological stress relative to that of those rats who received DJB; in the DJB group \( n = 6 \) that received the DJB operation, the gastric volume was maintained intact while the entire duodenum and the proximal jejunum were bypassed. The stomach was divided from the beginning of the duodenum. A length of 8 cm from the ligament of Treitz was measured to locate the site for the gastrojejunal anastomosis, which was performed using a 6-0 prolene suture. The continuity of the biliopancreatic secretions was reconstructed by anastomosing the biliary limb to the alimentary limb of the small bowel 12 cm distal to the gastrojejunal anastomosis in a Roux-en-Y fashion [6]. The matched-group normal control (NC; \( n = 6 \)) SD rats received regular chow, and the rats in DM, SO, and DJB groups were fed with a high-fat, high-sugar diet. All the rats were raised in an SPF environment, and they ate and drank freely. INS was not given to any group. The mental state, activity, hair, drinking, eating and urination of the rats were observed every day. One rat died within 24 h in the SO group. Mesenteric vascular thrombosis was its cause of death. A diabetic rat was assigned to the SO group to be the sixth rat needed to complete the group. After that, no more rats died. The rats were sacrificed by 1% sodium pentobarbital (intraperitoneal injection, 150 mg/kg) 12 weeks after surgery. The current study was conducted in accordance with the Principles of Laboratory Animal Care for the care and use of experimental animals, and the procedures were approved by the Chongqing Fuling Central Hospital Ethics Committee (flzxyy1203).

The INS-1 cell line was purchased from Shanghai Airui Biotechnology Co. The INS-1 cells were divided into the following three groups: group A was cultured with normal sugar content (11.1 mmol/L), group B was cultured with fluctuating high glucose (11.1 mmol/L alternating with 33.3 mmol/L for 24 h), and group C was cultured with fluctuating high glucose and exendin-4 (MedChemExpress; 100 nmol/L). All the groups were continuously cultured for 7 days in complete medium (RPMI 1640, 12% fetal bovine serum, and 1% bio-antibiotics) at 37°C in a 5% CO₂ incubator. The blood was collected from the rat' tail veins to test the blood glucose with a glucometer (One Touch LifeScan). Otherwise, the blood was collected from the retro-oral pelvicus of the rats under light ether anesthesia using the capillary tubes in Eppendorf tubes. The animals were fasted for 12–14 h to test their FBG, INS, and blood lipids. The serum was separated by centrifuge at 1,000 g for 15 min and collected and stored at −80°C. The total cholesterol, triglyceride (Beijing North Institute of Biotechnology), low-density lipoprotein (Shanghai Rongsheng Biotechnology), and TNF-α (Shanghai Biovalue) were measured using commercially available colorimetric diagnostic kits according to the instructions. The serum fasting INS (FINS) (Beijing North Institute of Biotechnology) was assayed by radioimmunoassay according to the instructions. For an oral glucose tolerance test (OGTT), 50% glucose injections of 40 mL were added to 60 mL of double distilled water and mixed well at a rate of 2 g/kg for filling the stomachs, and then a Johnson blood glucose meter (One Touch LifeScan) was used to set the time point (0, 30, 60, and 120 min) for checking the blood glucose.

At pre-operation, 2 weeks and 12 weeks after the operation, the OGTT test was performed, and the area under the OGTT curve was calculated.

\[
\text{AUC G(}\text{h mmol/L)} = (\text{G0} + \text{G30}) \times 0.5/2 + (\text{G30} + \text{G60}) \times 0.5/2 + (\text{G60} + \text{G120}) \times 1/2
\]

Note: G0, G30, G60, and G120 were 0, 30, 60, and 120 min of the blood glucose test.

The calculation formula for the IR index (HOMA-IR) was

\[
\text{HOMA-IR} = \text{FBG} \times \text{FINS}/22.5
\]

For an oral glucose tolerance test (OGTT), 50% glucose injection (11.1 mmol/L), group B was cultured with fluctuating high glucose (11.1 mmol/L) and exendin-4. All the groups were continuously cultured for 7 days in complete medium (RPMI 1640, 12% fetal bovine serum, and 1% bio-antibiotics) at 37°C in a 5% CO₂ incubator. The blood was collected from the retro-oral pelvicus of the rats under light ether anesthesia using the capillary tubes in Eppendorf tubes. The animals were fasted for 12–14 h to test their FBG, INS, and blood lipids. The serum was separated by centrifuge at 1,000 g for 15 min and collected and stored at −80°C. The total cholesterol, triglyceride (Beijing North Institute of Biotechnology), low-density lipoprotein (Shanghai Rongsheng Biotechnology), and TNF-α (Shanghai Biovalue) were measured using commercially available colorimetric diagnostic kits according to the instructions. The serum fasting INS (FINS) (Beijing North Institute of Biotechnology) was assayed by radioimmunoassay according to the instructions. For an oral glucose tolerance test (OGTT), 50% glucose injection (40 mL) were added to 60 mL of double distilled water and mixed well at a rate of 2 g/kg for filling the stomachs, and then a Johnson blood glucose meter (One Touch LifeScan) was used to set the time point (0, 30, 60, and 120 min) for checking the blood glucose.

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\]

Note: G0, G30, G60, and G120 were 0, 30, 60, and 120 min of the blood glucose test.

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Table 1. The changes of FBG, FINS, and body weight during the T2DM model being established

|                      | T2DM rats | Normal SD rats |
|----------------------|-----------|----------------|
| **FBG, mmol/L**      |           |                |
| Before STZ injection | 4.80±0.38 | 4.55±0.50      |
| 3 d after STZ injection | 10.58±2.46<sup>a</sup> | 4.13±0.39      |
| 1 wk after STZ injection | 15.28±1.86<sup>a</sup> | 4.22±0.62      |
| 2 wk after STZ injection | 16.21±1.79<sup>a</sup> | 4.50±0.35      |
| 4 wk after STZ injection | 16.54±2.41<sup>a</sup> | 4.43±0.64      |
| **Body weight, g**   |           |                |
| Before STZ injection | 241.33±5.58 | 242.83±6.05    |
| 1 wk after STZ injection | 248.67±6.60<sup>a</sup> | 266.83±7.00    |
| 2 wk after STZ injection | 254.67±6.89<sup>a</sup> | 288.67±7.79    |
| 3 wk after STZ injection | 260.33±7.48<sup>a</sup> | 312.00±7.01    |
| 4 wk after STZ injection | 265.89±7.67<sup>a</sup> | 341.00±6.29    |
| **FINS, μIU/mL**     |           |                |
| Before high-fat feeding | 7.57±0.37 | 7.48±0.36      |
| Before STZ injection | 16.25±0.78<sup>a</sup> | 7.54±0.35      |
| 4 wk after STZ injection | 12.10±0.52<sup>a</sup> | 7.71±0.33      |

<sup>a</sup>Compared with the normal SD rats, p < 0.05.

β-actin expression was used as the internal control for normalization. The primer pairs used for amplification were as follows:

- PKR-like endoplasmic reticulum kinase (PERK): 5′-GGCTTTGGGCTTCTTTGAG-3′ (forward), 5′-GGCTTTGACCTCCCGCAT-3′ (reverse);
- eukaryotic initiation factor 2α (eIF2α): 5′-TTACCTTTGACCTTCCCGAG-3′ (forward), 5′-GCTTTGACTTCCCGCATG-3′ (reverse);
- inositol-requiring enzyme 1 (IRE1): 5′-CAGGATGTTGAGTGACCGAATAGAA-3′ (forward), 5′-GGGCTTTGACCTCAGTTTTCCTC-3′ (reverse);
- BIP and CHOP: 5′-TGCTAGGAGCCAGGGCAATGAA-3′ (reverse), 5′-CGGACGGAGCCACCTTATAG-3′ (forward), 5′-TGACGTTGACATCCGTAAAGACC-3′ (forward), 5′-CGGACGGAGCCACCTTATAG-3′ (forward), 5′-TGCTAGGAGCCAGGGCAATGAA-3′ (reverse).

The transcriptional abundance was expressed as the fold increase above that of the control gene, as calculated by the 2^ΔΔCt method.

Western Blot Analysis

The pancreas tissues were homogenized in ice-cold modified RIPA buffer. The protein concentration was determined using a BCA assay. Equal amounts of protein per lane from each sample were separated on 10% sodium dodecyl sulfate polyacrylamide gels and transferred onto polyvinylidene fluoride membranes (Millipore, Billerica, MA, USA). The blocking was completed with 5% sheep serum blocking buffer by soaking at 37°C for 1.5 h. The membranes were probed overnight using the following antibodies: anti-β-actin (1:1,000 dilution; Abcam), and anti-β-actin (1:1,000 dilution; Proteintech, Chicago, IL, USA), anti-PERK (1:1,000 dilution; Cell Signalling), anti-IRE1 (1:1,000 dilution; Abcam), and anti-β-actin (1:1,000 dilution; Proteintech, Chicago, IL, USA). After being washed three times with Tris-HCl buffer solution, the immunolabelled membranes were incubated with goat anti-rabbit IgG secondary antibodies (1:1,000 dilution; Cell Signalling) at room temperature for 1 h. After incubation, the membranes were washed with Tris-HCl buffer solution again, and developed was added, and the bands were visualized by enhanced chemiluminescence (Millipore) and quantified with Quantity One software (Bio-Rad).

Statistical Analyses

All the results were expressed as the means ± SEM. The means of two groups were subjected to t tests, and multiple groups (≥3) of two groups were subjected to ANOVA; Tukey’s test was used in the post hoc analysis. A value of p < 0.05 was considered statistically significant. SPSS 17.0 statistical software was used for analysis.

Results

General Findings

Four weeks after the high-fat and high-sugar feeding, the FBG and weights showed no obvious difference between T2DM rats and normal SD rats, but the FINS in the...
T2DM rats was significantly higher than those of the normal SD rats \((p < 0.05)\) (shown in Table 1), which suggested that IR occurred in the T2DM rats. Three days after STZ injection, compared with the normal SD rats, the FBG in the T2DM rats had increased significantly \((p < 0.05)\) (shown in Table 1). One week after STZ injection, the weight growth in T2DM rats was lower than that of normal SD rats \((p < 0.05)\) (shown in Table 1). One week after STZ injection, the mean FBG value in the T2DM rats was greater than 11.1 mmol/L, and it became relatively stable 2 weeks after the STZ injection. Simultaneously, the T2DM rats showed decreased activity; messy hair; and significantly more drinking, polyuria, and bedding moisture; and thus, the bedding had to be changed daily instead of every 3 days. Four weeks after STZ injection, the weights of the T2DM rats were significantly lower than those of normal SD rats, and the FBG and FINS were significantly higher than those in the normal SD rats \((p < 0.05)\) (shown in Table 1). After the operation, the changes in body weights, blood glucose, lipids, FINS, HOMA-IR, and TNF-α were tracked as explained in our previous article (shown in Table 2) [18].

The first week after the operation, the body weights declined slightly in the SO and DJB groups (data not shown), but they exceeded their preoperative levels during the second week (still lower than group NC, \(p < 0.05\)) and kept rising slowly. The body weight increases in the SO and DM groups were slower than those in the NC and DJB groups after the operation, and the difference was significant 12 weeks after the operation \((p < 0.05)\).

Starting at the first week after operation, the FBG clearly decreased in the rats in the DJB group (data not shown).
shown), and it returned to normal from the second to 12th week, whereas the FBG in the SO and DM groups kept rising slowly \((p < 0.05)\). At the second week after operation, the total cholesterol, triglyceride, low-density lipoprotein, INS, TNF-\(\alpha\), and HOMA-IR in the DJB group were clearly lower than those in the SO and DM groups \((p < 0.05)\). These components were all restored at the 12th week, with no difference from those in group NC \((p > 0.05)\), whereas all of the factors in the SO and DM groups kept increasing \((p < 0.05)\) (shown in Table 2).

Two weeks after the operation, the AUC G in DJB group clearly declined relative to preoperative levels, and it was significantly lower than the levels in the SO and group DM groups \((p < 0.05)\); 12 weeks after the operation, the AUC G in DJB group was reduced more, but it was higher than that of the NC group \((p < 0.05)\); the AUC G in the SO and DM groups continued rising (shown in Fig. 1). These findings indicate that the promptly impaired glucose tolerance and IR improved significantly in the DJB group.

**Fig. 1.** OGTT experimental results (a) and the changes of AUC G (b) before and after operation in groups SO \((n = 6)\), DM \((n = 6)\), NC \((n = 6)\), and DJB \((n = 6)\). Preoperative, there has no difference of AUC G among groups SO, DM, and DJB; 2 weeks after operation, AUC G in group DJB declined clearly from preoperative, significantly lower than groups SO and DM \((p < 0.05)\); 12 weeks after operation, AUC G in group DJB reduced more but still higher than that of group NC \((p < 0.05)\). *Compared with group NC, \(p < 0.05\), **compared with group DJB, \(p < 0.05\).
GLP-1 Expression

The GLP-1 expression in the ileum cells under the mucosa in group DJB rats was strongly positive (++). In group NC, GLP-1 expression was positive (+). However, in groups SO and DM, only sporadic cells expressed GLP-1, the positive ratio was lower than 10% (×400). The GLP-1 IOD in group DJB rats was significantly higher than it was in the other three groups (p < 0.01), while it was higher in group NC than in groups SO and DM (p < 0.01). There were no obvious differences between groups SO and DM (p > 0.05).

Expression of PERK, eIF2α, and IRE1 mRNA

Twelve weeks after the operation, the amounts of mRNA expression in the ERS markers PERK, eIF2α, and IRE1 in SO and DM rats was significantly higher than those in the NC and DJB groups (p < 0.05), whereas no difference was observed between the DJB and NC groups (p > 0.05) (shown in Fig. 3).
Detection of PERK, p-eIF2α/eIF2α, and IRE1 proteins in the Pancreas

A Western blot analysis showed that PERK, p-eIF2α/eIF2α, and IRE1 protein expression significantly increased in the SO and DM groups 12 weeks after the operation, while they just slightly increased in group DJB (p < 0.05). There was no statistically significant difference between the groups DJB and NC (p > 0.05). Compared with group NC, p < 0.05, **compared with group DJB, p < 0.05.
Cell Viability and Apoptosis

The cell viability was measured by the MTT assay (shown in Fig. 5). The mean OD value in group B (0.3567 ± 0.0252) was significantly lower than that in group A (0.9333 ± 0.0451) (p < 0.05) and group C (0.5867 ± 0.0153) (p < 0.05). The apoptosis in group B was significantly higher than that in group A (p < 0.05) and group C (p < 0.05, shown in Fig. 6).

Detection of BIP and CHOP Proteins in INS-1 Cells

The ERS marker proteins BIP and CHOP were measured in the INS-1 cells by Western blot analysis (shown in Fig. 7). The BIP and CHOP proteins in group B were significantly higher than they were in groups A (p < 0.05) and C (p < 0.05).

Discussion

GBP can primarily contribute to reduced blood glucose in T2DM patients rather than to control weight [20–22], and surgical intervention within 5 years of diagnosis is associated with a high rate of long-term remission [23]. In severely obese patients with T2DM, the GBP resulted in better glucose control than medical therapy [24]. In obese patients with uncontrolled T2DM, significantly more patients achieved glycemic control through 12-month medical therapy plus bariatric surgery than with medical therapy alone [25]. However, the specific underlying mechanism is still unclear.

Effect of DJB on the Treatment of T2DM Rats

As a modified GBP procedure, DJB does not change the volume of the stomach or restrict food intake as it bypasses the duodenum and upper jejunum. In this study, we successfully set up a T2DM rat model, and the rat’s FBG decreased significantly 1 week after DJB. Because the operation trauma affected the rat’s food intake, the body weights declined slightly within the first week. However, 2 weeks after the operation, the rat’s body weights were restored and even exceeded preoperative levels with regained appetite, the FBG became normal, and HOMA-IR and AUC G clearly declined. Twelve weeks after the operation, the FBG and HOMA-IR levels remained normal, and the AUC G kept decreasing in the DJB group rats although their body weights exceeded those of the sham-operated and T2DM group rats, whose FBG, HOMA-IR, and AUC G levels kept raising. These findings suggest that the impaired glucose tolerance and IR improved in the DJB group after surgery. The slow increase in the body weights of the sham-operated and T2DM group rats was affected by diabetes. Therefore, it can be concluded that DJB can directly control the blood glucose of T2DM rats without depending on weight control. This result is consistent with Rubino’s conclusion that DJB can directly control T2DM rather than being secondary to weight loss or obesity in genetically diabetic rats [6] and consistent with Ramos reports that laparoscopic DJB can treat T2DM in patients with BMI <30 kg/m² [7].

There is a problem: DJB is a bariatric surgery, but why did the group DJB gain the body weight compared to the group SO and DM after DJB surgery? First, DJB surgery has no obvious effect on weight loss in nonobese patients and rats [10, 16, 26]. In this experiment, the 4-week-old male SD rats were fed on a short high-sugar and high-fat diet for 4 weeks and showed no growth advantage and no significant weight gain compared with SD rats on a normal diet (showed in Table 1). Second, after the DJB surgery, the disease of diabetic rats was controlled, so the weight loss caused by diabetes itself was controlled. The weight gain of rats in group SO and DM was not significant under the influence of diabetes. Third, DJB surgery did not alter gastric volume, so dietary intake was not affected.

GLP-1 Increased in T2DM Rats Receiving DJB

GLP-1 is considered one of the core mediating factors that control the postoperative blood glucose level in diabetic patients [27–29]. In vivo, GLP-1 is rapidly hydrolyzed by DPP-4 after the synthesis, to form an inactive GLP-1 (9–36) NH₂. As a result, the undamaged and bio-

![Fig. 5. The mean value of OD in group cultured with fluctuating high glucose (B), group cultured with normal sugar content (A), and group cultured with fluctuating high glucose and exendin-4 (C). Compared with group B, *p < 0.05.](image-url)
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active GLP-1 is only 10–20% of the total plasma GLP-1 [30]. Some researcher detected GLP-1 levels in the blood plasma through preset of the DPP4 inhibitor [13], and DJB causing increase of plasma GLP-1 has also been observed [16, 31]. But, GLP-1 has been partially degraded after entering the blood vessel, and the degradation degree may be different in different individuals and at different time points, which affect the accuracy of routine blood test results. In order to reduce errors and improve accuracy, drawing on the experience of other researchers, GLP-1 detection was prepositioned in the ileum cells where GLP-1 was produced [32]. Therefore, immunohistochemistry was used to investigate the expression of GLP-1 in L cells under the ileal epithelium.

We observed the sporadic positive expression of GLP-1 in the ileum L cells in SO and DM rats and the positive expression of GLP-1 in large amounts of L cells in DJB rats, which confirmed that DJB may regulate intestinal hormones, such as GLP-1, that play a role in the treatment. However, the specific mechanism requires further study. In addition, we found that the average absorbance value for GLP-1 expression in ileum L cells in the NC group is approximately 28% of that of the DJB group but higher than the levels in SO and DM rats. Thus, we spec-
ulate that T2DM itself will affect the expression of GLP-1 in ileum L cells as well. Cheong et al. [33] found that persistent hyperglycemia can reduce receptor expression via ERS, leading to a decrease in GLP-1 signal transduction.

We noted an interesting phenomenon in our research: as the GLP-1 increased, the β-cell apoptosis decreased in diabetic rats after DJB surgery, and theoretically, the INS should have increased as well. However, in this study, the opposite trend was found. We believe that the reason may be as follows: first, GLP-1 can not only protect β cells but it can also increase INS sensitivity and reduce IR, so the demand for INS was reduced. Second, there were more β cells in the pancreas of NC rats than in diabetic rats, but their INS levels were no higher than those in diabetic rats. In other words, β cells that are not at full capacity production may lead to decreased INS. Third, the presence of amylase or amylase-derived peptides in the gut reduces glucose absorption into the systemic blood circulation and simultaneously also limits INS release [34].

One study has shown no change in GLP-1 in rats after DJB [35], but the authors only measured GLP-1 levels at a single time point 10 days after the surgery. The authors stated that the measurement of GLP-1 levels as early as 10 days postoperatively might also explain why DJB did not affect GLP-1 secretion in their study. In fact, previous investigations in rodents suggest that elevation of GLP-1 levels may be a late phenomenon after DJB [13, 16, 31]. In these researches, GLP-1 levels were elevated at least 3 weeks after surgery.

**Inhibition of ERS by GLP-1**

ERS is well known to be involved in the development of diabetes [2, 36, 37]. We have confirmed that DJB has a hypoglycemic effect on experimental T2DM rats. Therefore, we supposed that ERS expression would be weakened in T2DM rats after DJB surgery. Our conjecture was confirmed by checking the ERS molecular marker PERK, eIF2α, and IRE1 mRNAs and PERK, p-eIF2α/eIF2α, and IRE1 proteins. We found that pancreatic ERS molecular marker PERK, eIF2α, and IRE1 expression decreased significantly in group DJB rats after their “nutritional excess” improved, which suggests that pancreatic ERS was inhibited following DJB surgery. This is consistent with the findings in other rat studies that DJB reduces ERS in the myocardium and liver as well. DJB and SG ameliorate diabetic cardiac dysfunction by inhibiting ERS (PERK-mediated pathway) in a diabetic rat model [38]. DJB alleviates hepatic ERS and decreases c-jun NH2-terminal kinase activity, which may contribute to improving blood glucose homeostasis after DJB operation [39].

So far, we have confirmed that DJB can enhance the expression of GLP-1 and inhibit ERS in T2DM rats. In addition, the DJB inhibition of ERS in T2DM rats is one novel finding of our research. Whether it inhibits ERS through GLP-1 is another question. We therefore performed the following experiment.

In a culture with fluctuating high glucose (11.1 mmol/L alternating with 33.3 mmol/L), the INS-1 cell viability significantly decreased, apoptosis increased, and the ERS molecular marker BIP and CHOP were significantly increased as well, which suggests that the ERS in INS-1 cells was induced. Simultaneously, when they were cultured with both fluctuating high glucose and exendin-4 (100 nmol/L) for 7 days, the viability of the INS-1 cells increased, apoptosis decreased, and the expression of BIP and CHOP were significantly decreased relative to the INS-1 cells cultured with fluctuating high glucose, which suggests that exendin-4 (GLP-1 analog) can inhibit ERS, reducing the apoptosis of β cell. This is consistent with the previous findings that GLP-1 receptor agonists reduced ERS in pancreatic cells [40, 41].

Circulating factors in patients with improved diabetes after metabolic surgery decreased the expression of BIP and CHOP, reduced β cell ERS, and exerted favorable effects on β-cell survival and function [42]. This is consistent with our research. But, it is not clear which factor is at work. It has been reported in the literature that bariatric surgery can improve IR and regulate blood glucose by regulating the expression of TNF-α, interleukin-6, C-reactive protein, bile acid, and other circulating factors in the serum of obese or diabetes patients [43–46]. In this study, we did find that TNF-α in the plasma of diabetic rats in the DJB group decreased significantly 12 weeks after surgery, while TNF-α in the plasma of diabetic rats in the DM group and the SO group continued increasing.

A lack of measure of plasma GLP-1 levels and a lack of knockdown experiments to suppress GLP-1 expression are the limitations of the present study. We will explore the IR factor released by the proximal small intestine and employ knockdown experiments to suppress GLP-1 expression in the coming experiments if funded. These experiments will explain how bypass surgery can improve T2DM by regulating ERS.

**Conclusion**

We concluded that DJB can be used to treat T2DM in T2DM rats. The mechanism may be that DJB stimulates the increased expression of GLP-1 on the far side of the
ileum, and then, GLP-1 inhibits the ERS in the pancreas, reducing the apoptosis of the β cells to achieve the treatment effect in T2DM rats.

**Statement of Ethics**

The current study was conducted in accordance with the Principles of Laboratory Animal Care for the care and use of experimental animals, and the procedures were approved by the Chongqing Fuling Central Hospital Ethics Committee (flxzyy1203).

**Conflict of Interest Statement**

The authors declare that there have no competing interests, and all the authors who read and approved the final manuscript should confirm its accuracy.

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**Author Contributions**

Xuan Chen, Zhen Huang, and Gang Liao were in charge of animal modeling and animal experiments, Xuan Chen and Qiang Zhang were in charge of the cell experiments, QingQiang Yang and ZiWei Wang were responsible for experimental design, data statistics, and article review, and the article was written by Xuan Chen.

**Data Availability Statement**

The datasets used or analyzed during the current study are available from Dr. Xuan Chen or the corresponding author on reasonable request.
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